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CHLOROPLAST DEVELOPMENT AND ASSOCIATED CHANGES IN PROTEIN COMPONENTS OF A BARLEY MUTANT

by

Ⓒ SWADESH JHAMB

A DISSERTATION
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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EDMONTON, ALBERTA
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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled 'Chloroplast development and associated changes in protein components of a barley mutant' submitted by Swadesh Jhamb in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Physiology and Biochemistry.

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To my parents

ABSTRACT

Development of plastids in the virescent mutant of Gateway barley was studied during greening of 7- and 10-day-old dark-grown seedlings and in seedlings grown in continuous light. Etioplasts of the mutant lacked crystalline prolamellar bodies, therefore, unlike the normal, development of grana and stroma lamellae could not occur by transformation of crystalline prolamellar bodies. However, prolamellar bodies were present in chloroplasts of the 4-day-old mutant when grown in continuous light of 1400 ft-c. Upon illumination the vesicles within etioplasts and young plastids of the mutant joined together to form grana lamellae. The formation of grana lamellae occurred before stroma lamellae.

Compared to normal barley the virescent mutant had many chloroplast abnormalities during early development. A striking abnormality was the presence of mitochondria and other cytoplasmic material in the plastids. From the electron micrographs it was inferred that the plastids entrapped these by an endocytotic-like process.

Two types of quasi-crystalline arrays which were different orientations of the same structure were observed within the stroma of chloroplasts. Morphologically they resembled cytoplasmic microtubules but were unlike them in their fixation properties and cold sensitivity.

When grown at 600 ft-c and 20°C the soluble leaf proteins separated into 21 discrete bands by electrophoresis. The most intense broad band of low mobility probably was fraction I protein and was present in 4, 6 and 8-day-old seedlings. At 4 days the amount of this protein in the mutant was small but it increased with age. By 8 days no differences were observed in the electrophoretic patterns of the two lines.

On treatment of chloroplast lamellae with 0.5% sodium dodecyl sulfate several proteins were separated electrophoretically from Gateway barley at 4, 6 and 8 days. Four-day-old seedlings of the mutant lacked some of the proteins but at 8 days the gel patterns of Gateway and its mutant were similar. When grown at 1400 ft-c and 22°C the mutant compared to Gateway lacked some proteins even at 8 days.

Under 600 ft-c and 20°C the mutant had about one-third as much chlorophyll as the normal at 8 days and under 1400 ft-c and 22°C it had only about one-fifth as much. Electron microscope studies of the leaves showed that Gateway barley had well developed chloroplasts under both growth conditions at 4 days, whereas the plastids of the mutant were not well developed at 4 or 6 days. By 8 days under the lower light intensity the mutant contained normal plastids but under the higher intensity the plastids were still not well developed. A relationship between chloroplast morphology and the proteins and pigments present was noted.

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LIST OF SYMBOLS AND ABBREVIATIONS

CE	chloroplast envelope
chl	chlorophyll
CPB	crystalline prolamellar body
DPIP	2,6-dichlorphenolindophenol
EDTA	Ethylenediaminetetraacetate
EM	Electron microscope
G	Grana
$KMnO_4$	Potassium permanganate
L	lamellae
M	Mitochondria
Mt	Microtubule-like structures
N	Nucleus
NE	Nuclear envelope
Os	Osmophilic granules
OsO_4	Osmium-tetraoxide
PB	Prolamellar body
PBT	Prolamellar body tubules
PL	Primary layers
PM	Plasma membrane
PSI	Photosystem I
PSII	Photosystem II
RuDP carboxylase	Ribulose-1,5-diphosphate carboxylase
S	Stroma
SDS	Sodium dodecyl sulphate
SDBS	Sodium dodecylbenzene sulphate

SL	: Stroma lamellae
St	: Starch grain
T	: Thylakoid
TEMED	: N,N,N^1,N^1-tetramethyl ethylenediamine
Tris	: tris(hydroxymethyl)aminomethane
V	: Vesicle
Va	: Vacuole
CW	: Cell wall
TS	: Thylakoid stacks

INTRODUCTION

Chloroplast mutations have been useful in the investigation of general metabolism, in the study of photosynthetic electron transport and in establishing the biosynthetic pathways of various pigments. The use of suitable mutants from higher plants, like the viable barley mutant under investigation, could help attain an understanding of the role of pigments, proteins and other constituents in the formation of chloroplast lamellae and in chloroplast morphogenesis.

Previous studies on the virescens mutant of Gateway barley have shown that the chloroplasts of the young seedlings were abnormal, contained large vesicles and were low in pigments. By two weeks, grana and stroma lamellae had developed and the seedlings contained 75% to 80% as much pigment as the normal (Maclachlan and Zalik, 1963). Pigment formation in the mutant was sensitive to temperature and light intensity (Miller and Zalik, 1965) and it was found that the deficiency in chlorophyll production was not due to an inadequacy of the chlorophyll precursor δ-aminolevulinic acid (Sane and Zalik, 1970). From studies of leucine-U-^{14}C incorporation it was noted that in the juvenile state incorporation by the mutant was less efficient than the normal and it was concluded that the mutation caused a partial inhibition in the synthesis of chlorophyll holochrome protein (Sane and Zalik, 1970). Although it is a single nuclear gene mutation (Walker *et al.*, 1963; Stephansen and Zalik, 1970) which manifests itself by low pigment levels and poorly developed chloroplasts, with time these correct themselves. Hence a detailed examination of the etioplasts during greening might reveal whether there is a defect in their morphogenesis. To investigate this possibility development in plastids of the normal and mutant was compared by electron microscopy. During this study microtubule-like structures were observed in the stroma and they were investigated further. One of the abnormalities noted in the mutant was regarded as an endocytolic-like process and forms a part of the electron microscope study.

To determine if any proteins were initially absent or deficient in the mutant and appeared during seedling development the soluble and lamellar proteins were examined electrophoretically. At each stage sections were taken for electron microscopy to determine the relationship between chloroplast structure and pigment and protein content.

LITERATURE REVIEW

CHLOROPLAST STRUCTURE

The chloroplasts of higher plants are lens-shaped and range from 5-10 μ in the long dimension and 2-3 μ in thickness (Kirk, 1967). The outer envelope consists of a double membrane. The envelope encloses the granular, proteinaceous mobile matrix - the stroma. Embedded in the stroma is the internal membrane system, made up of closed-flattened sacs called the thylakoids (Menke, 1962). Thylakoids are the structural units of the lamellar system and contain the pigments, most of the lipids, and about half of the protein of the chloroplast (Park and Pon, 1963). It has been established that morphologically distinguishable structures of the chloroplast, the stroma and the membrane system, have different biochemical functions. Isolated thylakoids can carry out photosynthetic electron transport and associated photophosphorylation, while the enzymes of the stroma are capable of reducing carbon dioxide (Arnon, 1959). Lipid synthesizing enzymes are also located in the stroma (Appelqvist *et al.*, 1968a).

Besides the lamellar system, the stroma contains osmophilic granules, starch granules, and pyrenoids, which respectively store lipid, starch and proteins. Pyrenoids have been frequently observed in the lower plants (i.e. *Chlorella pyrenoidosa*), and it has been suggested that they are specialized parts of the stroma (Gibbs, 1962). The pyrenoids of lower plants have a granular homogeneous matrix surrounded by starch deposits. Filamentous, fibrillar, and crystalline pyrenoids have also been observed (Evan, 1966; Bertagnolli and Nadakavukaren, 1970). Phytoferritin granules have also been found in the stroma (Hyde *et al.*, 1963). A mass of tightly packed fibrils has been observed in the stroma of the chloroplast of *Avena stavia* fixed in glutaraldehyde-OsO_4. Gunning (1965b) refers to these structures as "stroma centres". Besides the regular morphological features of the chloroplast of higher plants, fibrillar bundles of indefinite length were sometimes connected with the thylakoids and have been seen in the stroma of greening plastids (Bartels and Weier, 1967; Picket-Heaps, 1968; Sprey, 1968; Henningsen and Boynton, 1970).

The chloroplasts contain their own DNA, RNA and ribosomes. Sager and Palade (1957), in a study of the structure and development of the chloroplast of *Chlamydomonas reinhardi*, observed 100-150 Å granules in the stroma which appeared similar to the ribosomes of the cytoplasm. They suggested that these particles might be ribonucleoproteins. Various workers have observed similar particles in the chloroplasts of lower and higher plants. These particles have since been isolated and identified as ribosomes (reviewed by Krik, 1967). Brown and Gunning (1966) studied the shape and orientation of ribosome clusters in *Avena* plastids. In etioplasts, ribosomes were randomly distributed in the stroma. Furthermore, ribosome-like particles precisely distributed, one per cell unit of the lattice of the prolamellar body (description of PB is given in later part of the thesis) were observed by Gunning and Jagoe (1967).

Leaves treated with ribonuclease were devoid of these particles indicating that RNA was a major constituent of them. Polysomes were also seen in the chloroplast. In *Phaseolus vulgaris* whirl-like polysomes were observed attached to the membrane of stroma and grana lamellae (Falk, 1969), and in *Triticum aestivum* polysomes were arranged helically or in parallel unattached to the membranes (Bartels and Weier, 1967; Falk, 1969). This suggested that both free and membrane bound polysomes, as found in the cytoplasm, also exist in chloroplasts. It was concluded that in the chloroplast the membrane proteins of growing thylakoids were synthesized on the polyribosomes which were attached to these membranes (Falk, 1969). Plastids of mutants lacking ribosomes did not develop these membranes (Shumway and Weier, 1967; Goodenough and Levine, 1970). Moreover, from the observation that the RNA content of the plastid doubled during formation of the chloroplast from the etioplast in *Phaseolus vulgaris*, it was suggested by von Wettstein (1966) that this may indicate a doubling of the ribosomes prior to biosynthesis of the thylakoids.

The presence of DNA in the chloroplast was suggested in the early fifties on the basis of the Feulgen reaction. Later Ris and Plaut (1962) observed DNAase sensitive fibrils 25-30 Å thick in the chloroplasts of various plants. A number of workers have reported the existence of distinct electron transparent DNA containing regions in the chloroplasts of higher plants (Kislev *et al.*, 1965; Yokomura, 1967; Bisalputra and Bisalputra, 1967; Herrmann and Kowallik, 1970; Tewari, 1971). The fibrils were visible in samples double-stained with uranyl acetate and lead citrate. In *Ochromonas*, DNA fibrils were found to be organized in a ring-like pattern in the periphery of the chloroplast (Gibbs, 1968). In *Sphacelaria*, DNA of chloroplasts was also seen located towards the periphery on a distinct ring-like body named "genophore". A connection between genophores and the surrounding photosynthetic lamellar system has also been observed (Bisalputra and Bisalputra, 1969). The most satisfactory evidence for the presence of DNA was its actual isolation. Two forms of DNA, linear and reticulate, were isolated from spinach and *Acetabularia* chloroplasts. They were seen attached to the thylakoids under the EM (Woodcock and Fernandez-Moran, 1968). Fillipovich *et al.* (1970) isolated DNA by treatment of grana with 0.5% SDS. Single or multilayered cyclic ribosomes were liberated which were seen to be connected with portions of the DNA strand in EM preparations. They reported the length of the DNA strand was approximately equal to the perimeter of the thylakoids.

The lamellar systems of chloroplasts vary between species. In photosynthetic bacteria, the basic structure of the photosynthesizing system is found in the chromatophore which consists of a unit membrane surrounding an area of low electron density (Vatter and Wolfe, 1958). This resembles isolated stacked thylakoids of chloroplasts. Chromatophores contain bacterial chlorophyll and can carry out photophosphorylation. In some blue green algae, single thylakoids containing photosynthetic pigments are dispersed throughout the chromatoplasm. The single thylakoids of blue green algae are structurally intermediate between chromatophores and chloroplasts of other algae and higher plants (Niklowitz and Drews, 1956). In other blue green algae, branched thylakoids are observed and stacked thylakoids are present in green algae.

In higher plants between two and twenty small thylakoids are stacked together like piles of pennies to produce a granum. The large thylakoids extending between grana are called the stroma lamellae.

The molecular organization of the lamellae has been investigated using birefringence, low angle X-ray crystallography and their fine structure has been observed by sectioning, heavy metal shadowing, negative staining, and freeze-etching techniques (Park and Sane, 1971; Kirk, 1971). The use of these techniques has led to general agreement that a subunit structure exists within the membrane, however, many workers have postulated different arrangements for the chemical components constituting the lamellae and even workers who employed the same technique have not agreed in their views. Therefore, any existing controversy is due mainly to differences in interpretation of the results. Steinmann (1952) was the first to report the particulate character of the lamellae using chloroplast preparations shadowed with heavy metal. From studies of this type it was suggested that the membrane consists of a two dimensional sheet of subunits each about 50 Å in diameter (Frey-Wyssling and Steinmann, 1953). Later, an array of particles was observed by Park and Pon (1961) in metal shadowed preparations of thylakoids of sonically ruptured chloroplasts, and it was suggested that this might be the morphological expression of the photosynthetic unit of Emerson and Arnold (1932). Hence they named the particles "Quantasomes" (Park and Pon, 1963). In chemically fixed sections thylakoids appear as two dimensional sheets of globular subunits of about 90 Å in diameter with a light core and a dark rim (Weier and Benson, 1966). Kirk (1971), and Park and Sane (1971), argued that the subunits seen in these preparations were artifacts since it is difficult to explain how small subunits of 90 Å in diameter can be distinctly visualized in the 600 Å thick sections.

Mühlethaler *et al.* (1965) were the first to use the method of freeze-etching to study the subunit structure of the chloroplast membrane. Later the same technique was used by Branton and Park (1967). Although the experimental findings of both schools of workers are similar, they disagreed in their interpretation of the results. In freeze-etching the lamellae showed varying sizes of particles. According to Mühlethaler's group, the fracture plane would be formed at the membrane-medium interface and therefore the particles observed lie on the outer and lumen side of the thylakoids embedded in the lipid layer. On the other hand, Branton and Park (1967) have stated that in the frozen system the membranes provide a plane of natural weakness so that the breaks often proceed along inside the membrane face itself and the observed particles lie within the membrane. Later experiments with solvent extracted membranes, ferritin labelled red blood ghost cells and model lipid membranes seem to support Park's view (Park and Sane, 1971; Kirk, 1971). Two classes of particles produced due to the fracture plane were distinguished on the basis of size and were studied by Mühlethaler and by Park. The dimensions reported for these particles by the two groups are different.

The diagrammatic representation of the fracture faces of thylakoids that result from freeze-etching as presented by Park and Pfeifhofer (1969a) is given in Figure 1. The most accepted view is that the matching faces B and C are produced from the same internal fracture plane in freeze-etching. Face B is covered with particles of about 90 Å high and 175 Å in diameter, face C consists of particles of about 90 Å high and 120 Å in diameter. According to

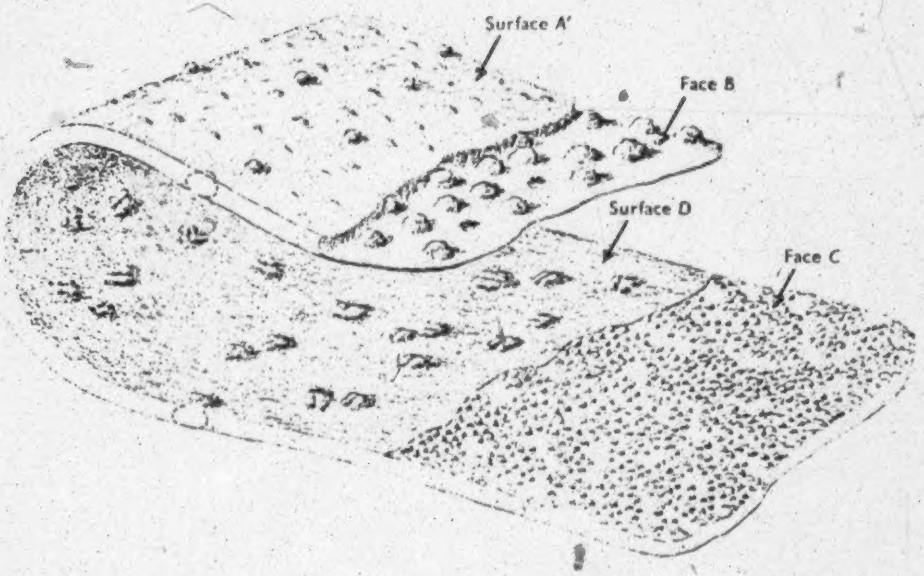

Surface A'
Face B
Surface D
Face C

Fig. 1. Diagrammatic representation of the deep etch surfaces (A', D) and fracture faces (B, C) commonly seen in thylakoids (according to Park and Pfeifhofer, 1969a).

Branton and Park (1967) particles of both sizes reside within the membrane. The 175 Å particles on face B correspond in their distribution to the quantasome particles observed in air-dried shadowed material but with the matrix removed. Since they were smaller in dimensions than quantasomes, Park and Pfeifhofer (1969b) named them "quantasome cores". The exterior (outer) face of the membrane, A, which becomes exposed in deep etching, is covered with a variety of proteins in unwashed lamellae. The interior face D is covered with particles of about 185 x 150 Å with the same periodicity as for the particles on fracture face B and they appear to possess 3 to 4 subunits.

Moudrianakis and co-workers (1967a and b, 1968) observed membrane-bound particles which were distributed either in a random or in a paracrystalline array. By shadowing and negative staining, they found that the size of the particles was dependent on the extent of shadowing. In negative staining, the size was 110 Å. The particles were removed from the thylakoid membranes with 1 mM EDTA and were identified as calcium dependent ATPase and they reported that these particles were identical to the originally described quantasomes of Park and Biggins (1964) seen in air-dried shadowed preparations.

Recently Kirk (1971) has reviewed the fine structure of thylakoids and has attempted to reconcile the data obtained from X-ray crystallography with that obtained from freeze-etching. Fig. 2 taken from Kirk (1971) represents a model for thylakoid membrane structure.

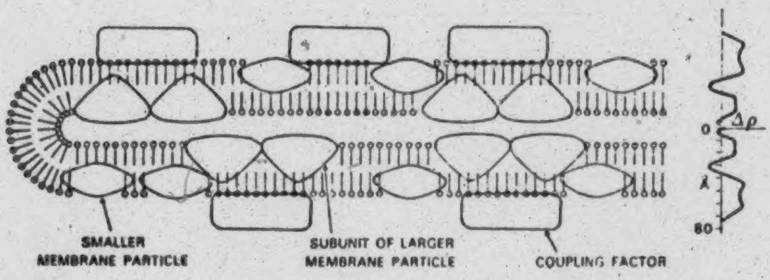

SMALLER MEMBRANE PARTICLE
SUBUNIT OF LARGER MEMBRANE PARTICLE
COUPLING FACTOR
0
Δρ
Å
80

Fig. 2. A model of thylakoid membrane structure which attempts to reconcile the data from X-ray diffraction with that obtained by electron microscopy. (According to Kirk, 1971)

The matrix of the membrane is a bilayer of lipid with polar groups extending outside and the hydrophobic chains of the bilayer are arranged inside. The bilayer of lipid is interrupted at places by two kinds of particles, supposedly proteins, observed by freeze-etching on fracture face B and C, Fig. 1. On the basis of distribution of electron density along a line normal to the plane of the thylakoid Kirk suggested that the 175 Å particles were nearer the inner membrane surface of the thalyakoid and the 120 Å particles were towards the outer side of the thylakoid. Thus the 120 Å particles of face C form a matrix surrounding the discrete 175 Å particles on face B. Moreover, he believes that the size of the particles must be smaller than the values given by some workers for the particles to fit into the membranes of thylakoids.

The membrane structure discussed so far holds true only for the membrane of partitions and has been observed by various workers in the chloroplasts of different plants. It is reported that the end granal membrane of the grana stack and the stroma lamellae which appear as single sheets of subunits upon chemical fixation contain only the smaller 120 Å particles. Sane *et al.* (1970) separated stroma and grana lamellae by fractionation and found that stroma lamellae possess PSI, while grana lamellae have both PSI and PSII as well as quantasomes (Park and Sane, 1971).

DEVELOPMENT OF CHLOROPLASTS

In lower plants such as algae and bryophytes, chloroplasts increase in number by division and are transmitted to daughter cells during cell division. Dividing chloroplasts have also been observed in higher plants (reviewed by Kirk, 1967). However, in higher plants it is not the mature chloroplast which is transmitted to the daughter cells by division. Instead colourless proplastids which are present in the meristematic cells have been seen dividing in electron-micrographs and are transmitted to daughter cells. Proplastids are undifferentiated bodies containing proteinaceous matrix and a few isolated small vesicles. These vesicles seem to bleb off from the inner enclosing plastid membrane since occasional invaginations from the inner plastid membrane have been noticed (von Wettstein, 1958; Mühlethaler and Frey-Wyssling, 1959: Laetsch and Stetler, 1965). As plastids grow the number of vesicles increases and their further development depends on different factors such as:

1, physical and chemical factors (light, temperature and nutrients)

2, internal factors (mutation of nuclear or plastid genes).

The ultrastructural organization of proplastids has been studied in different organs of several plant species by Tageeva *et al.* (1969). In proplastids of plant organs other than the leaf, the lamellar system is either absent or is in a rudimentary stage. Whereas in the leaf they develop eventually forming chloroplasts.

When germination of the seedlings is carried on in light at moderate temperature, proplastids of the leaf increase in size and their inner wall folds inwards to form flat vesicles or thylakoids. According to von Wettstein (1958) the most likely mechanism of thylakoid sac formation would be that the inner plastid membrane invaginates as a tubule and the tip of this tubule grows up to make a double membrane sheet. It is not certain whether these thylakoids are always joined at some point with the inner plastid membrane or whether eventually they are detached completely from it. These flat vesicles are electron transparent. As the plastids grow many more thylakoids are formed in the stroma, and start stacking together. The number of these stacks increases gradually and grana structures are formed. The grana are connected to one another by thylakoids. A few possible ways have been suggested for the multiplication of thylakoids. According to Menke (1964) the edge of the thylakoid might invaginate in the intrathylakoid space thus making two thylakoids. Wehrmeyer and Röbbelen (1965) studied this process using a pale green mutant of *Arabidopsis thaliana* which contains relatively few single thylakoids. From these studies they suggested that protrusions from a region of the thylakoid membrane might grow over the surrounding membrane to give rise to another thylakoid on top of the original one.

The development of chloroplasts during greening has been studied by various workers (von Wettstein, 1958; von Wettstein and Khan, 1960; Virgin *et al.*, 1963; Gunning, 1965a; Henningsen and Boynton, 1969, 1970). These studies involved experiments with dark grown seedlings which were then illuminated for different durations with light of various intensity and quality. If the seedlings were grown in the dark for 7 to 10 days the proplastids grew in size and began to differentiate. The vesicles which were formed by the invagination of the inner membrane of the proplastids aggregated to form crystalline prolamellar bodies (von Wettstein, 1958; von Wettstein and Khan, 1960).

The size of etioplasts depends upon the age of the dark grown seedlings, Generally they are smaller than chloroplasts and devoid of lamellae although in 10-day-old dark grown barley and bean primary layers were noticed radiating from the prolamellar body (von Wettstein, 1958; Henningsen, 1970; Henningsen and Boynton, 1969, 1970). The most characteristic feature of the etioplast is the presence of one to four crystalline centers also called crystalline prolamellar bodies which are joined to one another by thylakoids. The prolamellar bodies are made up of a three dimensional cubic lattice of interconnected tubules (von Wettstein, 1958; Virgin *et al.*, 1963; Gunning, 1965a). Gunning has carried out a detailed analysis of their structure by studying them in serial section in various planes. According to him, the membrane system of the crystalline prolamellar body is made up of six-armed "nodal units". The arms of the nodal unit are joined to the adjacent units in all directions giving rise to the cubic lattice, and the stroma material passes through the center of the spaces in the lattice. The cubic lattices are not very common (Mühlethaler, 1971). In the majority of the etioplasts the tetrahedral branched tubules of the prolamellar body are arranged in five membered or six membered conformations (Wehrmeyer, 1965). Osmophilic granules are also observed in the prolamellar body and it was suggested by Gunning and Jagoe (1967) that their presence initiated the formation of dislocations in the latter.

Combined evidence from phase contrast fluorescence microscopy (Boardman and Wildman, 1962), electron microscopy (von Wettstein and Khan, 1960; Henningsen and Boynton, 1970) and autoradiography (Lefleche *et al.*, 1972) has shown that protochlorophyllide was present in the crystalline prolamellar body of etioplasts and its accumulation coincided with the formation of the latter (von Wettstein and Khan, 1960; Klein *et al.*, 1964; Gunning and Jagoe, 1967; Henningsen and Boynton, 1967, 1969, 1970). The pigment was associated with a large protein and the entire complex, termed protochlorophyll holochrome (Smith, 1952), has been isolated and purified by ammonium sulphate fractionation. Its molecular weight was found to be 600,000 ± 50,000 and contained an average of one protochlorophyll per protein molecule (Boardman, 1962). In electron microscopy the complex appeared as a spherical molecule of 100 Å diameter. Negatively stained preparations of isolated prolamellar bodies showed them to be made up of macromolecules which according to Khan were similar in size to the protochlorophyll holochrome (Khan, 1968a). He suggested that the protochlorophyll holochrome was probably a structural component of the tubular membranes of prolamellar bodies. Furthermore, mutant barley, in which the protochlorophyll synthesis was blocked, failed to make prolamellar bodies (von Wettstein, 1961; Henningsen and Boynton, 1967; Boynton and Henningsen, 1967) suggesting that protochlorophyllide is required for the formation of the prolamellar body.

Various workers who have studied plastid development have separated the greening process into fairly distinct phases (Virgin *et al.*, 1963; Bogorad, 1967). (1) Protochlorophyllide transformation which involves the conversion of protochlorophyllide to chlorophyllide. (2) A lag phase during which there was no formation of additional pigment, the occurrence of which was dependent upon the age of the etiolated seedlings. It has been shown that the chlorophyll synthesis was limited due to slow formation of δ-amino levulinic acid (δ-ALA). The lag phase disappeared if the leaves were supplied with exogenous δ-ALA. Feeding with sugars also reduced the lag phase (Wolff and Price, 1966) and (3) the linear phase of chlorophyll synthesis began (Nadler and Granick, 1970). During this phase there was rapid formation of chlorophyll until net accumulation ceased.

Biochemical changes were accompanied by morphological changes and the three main steps were - tube transformation, tube dispersal and grana formation (von Wettstein and Khan, 1960; Virgin *et al.*, 1963; Henningsen and Boynton, 1970). Tube transformation and dispersal could occur after a short period of illumination followed by darkness, whereas grana formation could occur only in the linear phase of chlorophyll synthesis. When etiolated seedlings were exposed to light for a few minutes and sections of these were fixed in $KMnO_4$, structural rearrangement of the crystalline prolamellar bodies was observed. They lost their regularity and changed into an irregular network of tubules or vesicles (von Wettstein, 1958; Virgin *et al.*, 1963; Klein *et al.*, 1964). Gunning and Jagoe (1967) while studying the development of plastids of oat seedlings fixed in glutaraldehyde-OsO_4 observed that the prolamellar body was disorganized when illuminated for 5 min (intensity 750-1000 ft-c) and formed a sheet of double lamellae. They did not observe the formation of vesicles or tubules. However, Henningsen and Boynton (1970) observed tube dispersal in barley plastids, fixed in glutaraldehyde-OsO_4. Therefore, the differences observed during transformation of the crystalline prolamellar body are probably not due to the fixatives used contrary to the view expressed by Kirk (1967).

The frequency of structural changes in the prolamellar body on exposure to light depended on the wavelength and energy supplied. A rough parallelism was observed between the proportion of etioplasts which had undergone structural changes and the proportion of protochlorophyllide converted to chlorophyllide. Tube transformation and protochlorophyllide conversion had similar action spectra and energy requirements (Klein *et al.*, 1964; Virgin *et al.*, 1963). Khan (1968b) has reported that a single brief light flash of 1 ms can elicit both processes. Red and blue light were found effective whereas green light was ineffective. In a few instances crystalline prolamellar bodies were observed although all the protochlorophyllide had been converted into chlorophyllide. Tube transformation of these crystalline prolamellar bodies could take place in the dark after conversion of all the pigment. When 7-day-old etiolated barley plastids were exposed to dim light of 2 ft-c tube transformation and dispersal of initial prolamellar bodies occurred. New prolamellar bodies, at several places along with primary layers, were formed on longer illumination (Henningsen and Boynton, 1970). From these results the authors concluded that pigment conversion and tube transformation were not the primary process since both occurred in dim light. Both processes were often synchronized but could be separated by changing the physiological state of plastids or conditions of illumination (Henningsen, 1970).

In the second step tubes dispersed and moved throughout the stroma and formed regularly spaced primary layers (Virgin *et al.*, 1963). If all the protochlorophyllide of the prolamellar body was converted into chlorophyllide the dispersal process could occur in darkness. The dispersal process did not require chlorophyll formation since it took place in the lag phase of chlorophyll synthesis (Klein *et al.*, 1964). This reaction had a considerably higher energy requirement than tube transformation. The action spectrum of tube dispersal revealed that only blue light was highly effective. Chlorophyll a, phytochrome, β-carotene, and xanthophyll were therefore ruled out as energy absorbing pigments for this process (Henningsen, 1967). The rate of dispersal of tubes increased with increasing light intensity. In high light the process could occur in a few minutes.

This suggested that the dispersal process was not the cause of the lag phase. Dispersal was found to be a temperature dependent process, since it did not occur at 0° or 10°C and was maximal at 30°C (von Wettstein, 1967; Henningsen, 1970).

The changes in the chloroplast structure during greening were associated with shifts in the absorption maxima of the pigments, and a few hypotheses have been put forward to explain the shifts. The main portion of the protochlorophyllide accumulated in the etioplast had an *in vivo* absorption maximum of 650 nm and a small portion had an *in vivo* absorption maximum of 632 nm. Only protochlorophyllide 650 was photoconverted to chlorophyllide (Wolff and Price, 1957). The chlorophyllide formed absorbed initially at 678 nm but shifted rapidly to 683 nm (Gassman *et al.*, 1968). Various workers believed that this change in the absorption maxima was due to a conformational change in the holochrome protein (Gassman *et al.*, 1968; Henningsen, 1970) and the change was accompanied by the tube transformation seen by electron microscopy (Khan, 1968b). The absorption maximum of chlorophyllide 683 shifted back to 672 in a period of 10 min - "the Shibata shift" (Shibata, 1957; Akoyunoglou and Siegelman, 1968), this has been correlated to the dispersal of the prolamellar body tubules into primary layers. Henningsen (1970) suggested that this spectral shift was due to disaggregation of the subunits of holochrome and relocation of chlorophyll in the protein-lipid interface because the absorption maximum of artificially dispersed chlorophyll in a water-lipid interface was 672 nm (Chapman and Fast, 1968). Klein (1962) and Sironval *et al.* (1965) suggested that phytylation of newly formed chlorophyll was the cause of this spectral shift. Smith *et al.* (1959) did not observe the spectral shift in 3 albina mutants which lacked phytylation capacity. On the contrary, in freeze-thawed leaves the spectral shift was observed without an increase in phytylated pigment which was taken as evidence that phytylation was not the cause of the spectral shift (Henningsen, 1970).

Multiplication of the lamellar discs to form grana paralleled the rapid increase in chlorophyll synthesis in barley plastids (Virgin *et al.*, 1963). The pores of the membranes in oat plastids disappeared and grana formation occurred in the linear phase of chlorophyll synthesis. From rough estimates of perforated thylakoids it was concluded that there was no large area of membranes synthesized before the linear phase of greening (Gunning and Jagoe, 1967; Henningsen and Boynton, 1969). It was observed that in the absence of chlorophyll the discs were formed but they did not aggregate to form grana (von Wettstein, 1958). De Greef *et al.* (1971) observed unfused stacks of thylakoids in bean leaf plastids during 60 hr illumination in far-red light. These thylakoids fused to form grana only when placed in white light. From the results they concluded that grana formation was not solely a function of chlorophyll accumulation but invol energy light reaction.

Etioplasts were not able to carry out electron transport, light dependent ATP synthesis, and CO_2 fixation. Photochemical activity of the chloroplasts and capacity for light driven electron transport and oxygen evolution developed simultaneously with the formation of grana structure. Addition of chloramphenicol or actinomycin D at the lag phase or at various times during greening in the linear phase arrested further greening even when illumination was continued, and this suggested that the chlorophyll accumulation in the linear phase was dependent upon continuous protein synthesis and upon DNA dependent RNA synthesis (Bogorad and Jacobson, 1964; Margulies, 1962). von Wettstein (1967) noted that DNA content remained practically constant during the entire process of chloroplast development. There was an increase in RNA content which doubled during the same time period in which there was a steep increase in chlorophyll synthesis and grana formation. Nadler *et al.* (1972) concluded that development of photosystem II was limited by the synthesis of proteins and not by chlorophyll synthesis.

From studies using carotenoid-deficient mutants of *Helianthus annuus* and *Zea mays*, Walles (1967) and Bachmann *et al.* (1967) concluded that carotenoids were necessary to stabilize the aggregation of discs in the grana but were not required for tube transformation and tube dispersal. A number of studies have indicated that lipid synthesis is necessary for grana development (Kirk, 1967).

It is well known that photosynthetic CO_2 fixation in higher plants and algae operates via the Calvin cycle. Recently, another pathway for CO_2 fixation, the C_4-dicarboxylic acid pathway, has been found to be important in tropical grasses. The primary photosynthetic product formed is a C_4-dicarboxylic acid instead of phosphoglyceric acid and these plants have higher levels of PEP carboxylase. On the basis of the mode of CO_2 fixation higher plants are divided into two main categories, species which utilize the Calvin cycle designated as C_3 plants and species which utilize the C_4-dicarboxylic pathway designated as C_4 plants. C_4 plants like sugar cane, sorghum and corn contain dimorphic chloroplasts but C_3 plants do not. The chloroplasts of the bundle sheath cells of C_4 plants have parallel lamellar sheets extending throughout the stroma and have the ability to store starch, whereas mesophyll chloroplasts are smaller in size and contain grana (Laetsch, 1968; Laetsch and Price, 1969). These chloroplasts contain a peripheral reticulum which was initially considered to be a unique structural feature of C_4 plants (Laetsch, 1970), however there are some reports of chloroplasts from C_3 plants also having a peripheral reticulum (Gracen *et al.*, 1972).

The structural dimorphism of the chloroplasts has been associated with their function. It was shown that the C_4-dicarboxylic acid pathway occurred exclusively in mesophyll chloroplasts of C_4 plants whereas the pentose phosphate cycle occurred in bundle sheath chloroplasts (Slack *et al.*, 1969).

AUTONOMY OF CHLOROPLASTS

The first indication for the genetic continuity of chloroplasts came from the analysis of variegated plants and from studies of interspecific crosses in higher plants. This subject was extensively reviewed by Kirk (1967). Another line of evidence supporting genetic autonomy of the chloroplast came from the experiments of Provasoli *et al.* (1951) on *Euglena gracilis*. When they grew the algae in the presence of 40 μg/l of streptomycin the cells bleached permanently after several generations and they never regained chloroplasts. Thus the nucleus of the cell was unable to direct the synthesis of new chloroplasts. The results showed that treatment destroyed the self-contained genetic system of the chloroplasts.

It is now generally accepted that chloroplasts have some degree of autonomy. These self-replicating organelles contain not only DNA and RNA but also the complete apparatus for synthesizing some of their proteins. Some evidence for the presence of DNA in chloroplasts came from light microscopy of Feulgen-stained material, as well as electron microscopy and autoradiography. In the last decade many reports have appeared on the isolation of DNA and its characterization but it now appears that only a few workers have actually isolated chloroplast DNA (Kirk, 1963, 1971a;

Tewari and Wildman, 1966; Tewari, 1971). Chloroplast DNA of higher plants and algae was different than nuclear DNA in its guanine/cytosine ratio, buoyant density and in its lack of 5-methyl cytosine. It was also different from mitochondrial DNA (Kirk, 1971). From studies with *Acetabularia* it was shown that the synthesis of chloroplast DNA is independent of the nucleus (Gibor, 1967). Tewari and Wildman (1967) reported incorporation of thymidine into DNA. The isolated DNA with label appeared at the same place on CsCl gradients as the native DNA of chloroplasts. Its hybridization with chloroplast DNA indicated that it was synthesized on the pre-existing DNA of chloroplasts and showed that chloroplasts had their own DNA polymerase.

The amount of DNA in the chloroplast of algae and higher plants is comparable to the amount present in prokaryotes. However, from heat denaturation and renaturation studies it appeared that the amount of DNA in the chloroplast was twenty to thirty times more than the amount of information present. According to Kirk (1971a) DNA of chloroplasts could code for 180 to 300 proteins containing an average of 300 amino acids. More than one DNA-containing region has been observed in higher plant chloroplasts and it has been suggested that they have multiple copies of DNA. Chloroplasts contain RNA and hybridization studies indicated that chloroplast DNA contains cistrons for chloroplast ribosomal RNA (Kirk, 1967). DNA dependent RNA polymerase has been found in the chloroplasts of many plants (Tewari, 1971).

There are a number of reports available on the isolation of 70S ribosomes from the chloroplasts of lower and higher plants which were active in protein synthesis (Sissakian *et al.*, 1965; Hadziyev and Zalik, 1970; Jones *et al.*, 1973). Like bacterial ribosomes the chloroplast ribosomes were selectively inhibited by chloramphenicol but not by cycloheximide (Jones *et al.*, 1973). Taking advantage of the selective inhibition by these antibiotics Smillie *et al.* (1968) studied the chloroplast proteins synthesized on 70S ribosomes of chloroplasts and 80S ribosomes of cytoplasm. They found that the chloroplast did not synthesize all of its proteins but some were synthesized by the 80S ribosomes.

On the basis of the present evidence about the genetic apparatus and its role in the formation of chloroplasts, the most popular hypothesis is that chloroplasts at one time were free living prokaryotic organisms like bacteria and became endosymbionts during the course of evolution (Raven, 1970). Since chloroplasts possess a certain degree of autonomy and they have their own DNA and ribosomes it is reasonable to expect that they should have the ability to duplicate. Chloroplasts of algae have been seen multiplying by fission and during ensuing cell division they were transmitted to the daughter cells. Dividing proplastids and chloroplasts have also been observed in higher plants (Kirk, 1967). Ridley and Leech (1969) and Gilies and Safaris (1971), while investigating the survival capacity of chloroplasts *in vitro* in *Vicia faba* and algae respectively reported division of the chloroplasts in an artificial environment. However, the morphological changes which occurred during the division of these isolated chloroplasts were not investigated. Cavalier-Smith (1970) has presented electron microscope evidence that in *Chlamydomonas reinhardi* two chloroplasts contributed from two parent cells fused as do nuclei to form a single zygotic chloroplast. These observations support the theory that plastids do not arise *de novo*, however, it is not clear how they are transmitted from one generation to the next. Bell and Mühlethaler (1962) proposed that plastids may not always arise from preexisting plastids. From their electron microscope and autoradiographic experiments Bell and Mühlethaler (1964) claimed that plastids of *Pteridium* were eliminated during oogenesis and were formed *de novo* from the evagination of the egg nucleus. However, Jensen (1965) did not observe degeneration of plastids and mitochondria in cotton plants. They were present at all stages of development in the egg cell. The question about the origin of plastids is therefore still unresolved and the EM observations by themselves have not so far provided convincing evidence for *de novo* synthesis of plastids.

CHLOROPLAST MUTANTS

Nuclear and cytoplasmic genes control the morphology and the function of chloroplasts. Gene mutations may cause blocks in the synthesis of some chemical components like pigments, proteins and lipids which are required for chloroplast formation. Deficiency of the pigments can effect general metabolism, a particular function and/or differentiation of chloroplasts. These mutations have been very useful in the investigation of the biosynthetic pathways of various pigments and in understanding their morphogenetic role in the formation of chloroplast lamellae.

Several excellent reviews have appeared recently on chloroplast mutants (Kirk, 1967; Levine, 1969; Walles, 1971) which affect photosynthetic enzymes, pigments and general metabolism. A number of nuclear mutants of *Chlamydomonas reinhardi* were deficient in the enzymes of the photosynthetic electron transport chain or in enzymes required for the Calvin cycle. The structure of their chloroplasts appeared normal under the electron microscope which suggested that the lack of one enzyme did not cause drastic changes in chloroplast structure (Goodenough and Levine, 1969).

Investigation of some chlorophyll-deficient mutants of algae and higher plants demonstrated that some reactions in the biosynthesis of chlorophyll were under nuclear control. Granick (1951) studied mutants of *Chlorella* which had various blocks in the synthesis of chlorophyll. On the basis of accumulation of specific porphyrin intermediates the biosynthetic steps in chlorophyll formation were determined. A number of barley mutants have been studied by von Wettstein *et al.* (1971). Mutants belonging to gene loci *f*, *g*, *h*, and *u* failed to synthesize protochlorophyll, and it was suggested that the four loci controlled sequential steps in the synthesis of protochlorophyllide prior to the formation of Mg-protoporphyrin. These mutants did not form prolamellar bodies (Boynton and Henningsen, 1967). *xantha-1* seemed to control the step between Mg-protoporphyrin and protochlorophyllide. A mutant controlling transformation of protochlorophyllide to chlorophyllide has not yet been found in barley. However, a mutant of *Arabidopsis thaliana* synthesized protochlorophyllide but was unable to convert it to chlorophyllide (Röbbelen, 1956).

The barley mutant *albina-17* and *Xantha-j* were blocked in the dispersal of tubes as well as in the "shibata shift", whereas *xantha-m* and *xantha-a* were blocked only in the dispersal of tubes. Further analysis of these mutants might provide some answers to the relationship between the two processes (von Wettstein, 1971). *Xantha-b*, *e*, *c* and *d*, all prevented the formation of grana.

Lipid metabolism was also studied in these mutants (Appelqvist *et al.*, 1968b). Qualitatively the fatty acid composition of these mutants was similar to that of the wild type. However, the incorporation of acetate into phospho-, sulfo- and galactolipids was suppressed by the *xantha-f* mutants. *Xantha-m* incorporated higher amounts of labelled acetate into linolenic acid of monogalactosyl diglyceride than the wild type. Since the quantity of this lipid in *xantha-m* was higher than in the wild type the authors concluded that the *xantha-m* locus had a controlling function in the synthesis of monogalactosyl diglyceride. Although *xantha-a* contained a small amount of chlorophyll it was unaffected in lipid formation.

The function of chlorophyll b in plastid development is unknown. Goodchild *et al.* (1966) studied a chlorophyll b-deficient mutant of barley. The plastids contained smaller and fewer grana. On the other hand, the chlorophyll b-deficient mutant of *Arabidopsis* has normal chloroplast structure (Veleminsky and Röbbelen, 1966).

The role of carotenoids in the formation of chloroplast structure in higher plants has been investigated by using carotenoid-deficient mutants of *Zea mays* (Bachmann *et al.*, 1967) and *Helianthus annuus* (Walles, 1965, 1966, 1972). The plastids of the dark grown seedlings of these mutants formed prolamellar bodies and ribosomes. There was no block in the formation of chlorophyll under ordinary light conditions but the chlorophyll formed was destroyed by photooxidation. An *albina* mutant of *Helianthus annuus* lacked β-carotene and xanthophyll, whereas a *xantha* mutant contained xanthophyll and lutein but lacked β-carotene. In low light intensity structural differentiation of plastids proceeded until a few grana were formed. The grana in *xantha* were larger than in *albina*. Grana were destroyed soon after formation due to photooxidation of chlorophyll. The degenerate plastids were irregularly shaped and contained swollen vesicles. The *albina* mutant accumulated a colorless compound, phytoene, which is a precursor of carotenoids. In *Zea mays* lycopene and zeta-carotene were present in two mutants but no other carotenoids were found. There was poor development of thylakoids and grana. The latter were not stable and were destroyed quickly upon illumination like *albind* and *xantha* of *Helianthus*. It was concluded that carotenoids did not play any role in the formation of the prolamellar body, thylakoids or grana because they were formed in dim light, but carotenoids were necessary for the protection of chlorophyll (Walles, 1965, 1966; Bachmann *et al.*, 1967). According to von Wettstein (1961) the plastids of carotenoid-deficient mutants of barley contained groups of large globuli, which probably consisted of unusable pigments.

Recently, Walles (1972) reported that plastid ribosomes of carotenoid-dificient *albina* and *xantha* mutants of *Helianthus annuus* were destroyed upon illumination. According to him ribosomes were sensitive to light normally absorbed by β-carotene. Millerd *et al.* (1969) studied a temperature sensitive mutant of maize and found that when grown at 27°C the plastid structure was similar to its wild type. However, when grown in light or dark at 15°C its plastids were abnormal. The etioplasts contained less protochlorophyll but ribosomes were present. Plastids of light grown seedlings contained lower amounts of carotenoids and chlorophyll. The membranes and ribosomes of plastids were destroyed due to extreme sensitivity to light.

Several chloroplast mutants which appeared to be defective in their general metabolism were shown to be deficient in a single metabolite. *Albina-7* requires aspartic acid and *xantha-23* requires leucine to form normal green chloroplasts (Walles, 1963). Boynton (1966) studied the ultrastructure of thiamine requiring tomato plants. Their plastids contained grana in early stages of development, which broke down into a diffuse substructure during bleaching of the leaves. When supplied with thiamine the chloroplasts regained their normal structure.

A single gene, virescens mutant of barley has been investigated by Zalik and co-workers (1963, 1965, 1968, 1970, 1971). The juvenile seedlings were low in pigments and the plastids contained large vesicles. An increase in the pigment content occurred with age and the plastids formed normal grana and stroma lamellae (Maclachlan and Zalik, 1963). It was suggested that the mutation had caused an alteration in lipoprotein during early stages of development. Chlorophyll accumulation in this mutant was found to be sensitive to light quality, intensity and temperature. The highest chlorophyll accumulation occurred in green light. A large Q_{10} value was obtained for chlorophyll accumulation in the mutant in the temperature range which indicated that a highly temperature sensitive reaction was involved in the mutation (Miller and Zalik, 1965). Sane and Zalik (1970) found that production of chlorophyll precursor was not affected in the mutant but it had a low protein synthesizing capability at early stages of development which in turn affected chlorophyll production. The primary biosynthetic reaction block in the mutant is still unknown, however, it was proposed that the mutation affected the production of chlorophyll indirectly by affecting the synthesis of chlorophyll holochrome protein.

A virescent mutant of *Zea mays* has been studied (Chollet and Paolillio, 1972; Chollet and Ogren, 1972) which resembles in many of its characteristics the virescent mutant of Gateway barley. Before greening its seedlings were low in pigments and plastids contained rudimentary vesicular and lamellar components. The CO_2 fixation rates were low and the activity of RuDP-carboxylase was markedly reduced along with some other enzymes. Upon greening the pigment content, plastid structure and enzymatic activities of plastids became normal.

A number of reports show that chlorophyll-deficient mutants of higher plants have higher photosynthetic capacity at the same (Benedict and Kohel, 1970; Keck *et al.*, 1970b) or higher light intensity than the wild type (Schmid, 1967; Clewell and Schmid, 1969; Highkin *et al.*, 1969) on the basis of chlorophyll content. Schmid (1967) investigated the photosynthetic capacity and the lamellar structure in various chlorophyll-deficient plants of *Nicotiana tabacum*. The plastids of virescent 402 contained fully developed grana in spite of the chlorophyll deficiency but showed impaired rates of photosynthesis. An increased capacity for photosynthesis was found in plastids of healthy *aurea* mutants which had widely separated single lamellae. However, variegated tobacco contained normal plastids in green parts and separated single lamellae in yellow green parts. Both types of plastids were incapable of photosynthesis. These results suggested that the arrangement of the lamellae in grana or as single layers has no effect on the capacity for photosynthesis. The plastids of chlorophyll-deficient *Lespedeza procumbens* contained fewer thylakoids but had a higher photosynthetic capacity especially at higher light intensity (Clewell and Schmid, 1969). In a pigment-deficient, single gene, virescent mutant of cotton the rates of synthesis of RuDP-carboxylase, CO_2 fixation and photosynthetic capacity per molecule of chlorophyll were higher than in the wild type (Benedict and Kohel, 1968, 1969, 1970). The young chloroplasts of this mutant lacked lamellar aggregation but mature leaves formed normal chloroplasts. The plastids of a pea mutant deficient in chlorophyll contained fewer lamellae per plastid and fewer lamellae per granum than normal but on a chlorophyll basis, rates of CO_2 fixation and Hill reaction activity in the mutant was twice as high as in the normal (Highkin *et al.*, 1969).

PROTEINS OF CHLOROPLASTS

Soluble proteins.

Wildman and Bonner (1947) observed that 70-80% of the soluble leaf protein was electrophoretically homogeneous and had an S value of 18. They designated this high molecular weight leaf protein as Fraction I protein to distinguish it from other proteins in the leaf extract. Later it was shown that this component was localized in the chloroplast and that it was one of the major components of the water soluble proteins (Lyttleton and Ts'O, 1958). Fraction I protein is a large protein with a molecular weight of 500,000-570,000.

Various workers have succeeded in purifying a carboxylase enzyme, identified as ribulose-diphosphate carboxylase from spinach and rice leaves and it had physical properties similar to Fraction I protein (Weissbach *et al.*, 1956; Mendiola and Akazawa, 1964). About the same time a highly purified protein-protochlorophyll complex was obtained from etiolated plants and it had similar properties to those of Fraction I protein and ribulose-diphosphate carboxylase (Boardman, 1962; Trown, 1965). It has not been reported whether holochrome preparations have RuDP carboxylase activity. Akoyunoglou *et al.* (1970) isolated RuDP carboxylase and protochlorophyllide holochrome from etioplasts of *Phaseolus vulgaris* and reported that the two proteins were not identical. When the proteins were extracted in tris-HCl buffer RuDP carboxylase was released, however, protochlorophyllide holochrome was extracted on addition of Triton X-100.

Although there has been general agreement that Fraction I protein is ribulose-diphosphate carboxylase, Anderson *et* *al.* (1968) isolated a few carboxylases from some microorganisms which were significantly different in molecular weight from Fraction I protein. Therefore Kawashima and Wildman (1970) opted to retain the term Fraction I protein to designate exclusively the high molecular weight protein which has been found wherever chlorophyll a is present.

Fraction I protein from rice leaves, when separated on Sephadex G-200 had ribulose phosphate isomerase, phosphoribulokinase and ribulose-diphosphate carboxylase (Mandiola and Akazawa, 1964). However, Thornber *et al.* (1966) showed that the isomerase and kinase activity could be removed from Fraction I protein of *Beta vulgaris* by column chromatography to give a purified protein which appeared as a single peak on acrylamide gel electrophoresis. Under the electron microscope Fraction I protein from chinese cabbage chloroplasts appeared as cubic particles 120 Å on a side (Trown, 1965).

Rutner and Lane (1967) found that on treatment with SDS, RuDP carboxylase from spinach dissociated into two distinct subunits which had different electrophoretic mobilities, sedimentation velocities and amino acid compositions. By SDS gel electrophoresis the molecular weight of the larger subunit was 55,800 and the smaller subunit was 12,000. Moon and Thompson (1969) estimated the molecular weight of small and large subunits as 16,000 and 54,000 respectively.

The site of synthesis of Fraction I protein has been investigated by the use of antibiotic inhibitors. Chloramphenicol binds chloroplast ribosomes and inhibits protein synthesis by chloroplasts whereas cycloheximide has no effect on protein synthesis by chloroplasts but inhibits protein synthesis of cytoplasm. Experiments with algae and higher plants have shown that the accumulation of RuDP carboxylase was strongly inhibited by chloramphenicol and slightly by cycloheximide (Margulies, 1971; Smillie *et al.*, 1967; Ireland and Bradbeer, 1971). Margulies and Parenti (1968) has reported incorporation of amino acid in Fraction I protein in isolated chloroplasts of *Phaseolus vulgaris*. These results suggested that the enzyme is synthesized on the chloroplast ribosomes. Criddle *et al.* (1970) reported that in barley the synthesis of smaller subunits was inhibited by cycloheximide whereas chloramphenicol markedly inhibited the synthesis of the large subunit. From these results they suggested that the smaller subunits may be formed on cytoplasmic ribosomes and large subunits may be formed on chloroplast ribosomes. Hoober (1972) reported that in a *y-1* mutant of *Chlamydomonas reinhardi* the synthesis of both subunits of RuDP carboxylase was inhibited by chloramphenicol. According to him the enzyme is synthesized in the chloroplast and mRNA for the enzyme is made in the nucleus.

LAMELLAR PROTEINS

Menke and Jordan (1959) obtained lamellar protein by extraction of chloroplast lipids and pigments with methanol and ether, and soluble proteins with NaOH and NaCl. It was found that lamellar structural proteins make up 44 to 54% of the total protein of the chloroplast (Menke, 1962). Park and Pon (1961) found that purified preparations of spinach chloroplast lamellae had 48% lipid and 52% protein. Investigation of the protein of the lamellae has been hampered by the insolubility of the protein at physiological pH. In the last decade attempts have been made to isolate and characterize the lamellar proteins of the chloroplast. Weber (1962) solubilized lamellar structural protein in 99% formic acid from chloroplasts of *Antirrhinum majus*. The lipids and carbohydrates were separated and the formylated protein moved as a uniform 5.9 S peak in the ultracentrifuge. Biggins and Park (1965) solubilized the lamellae of spinach chloroplasts in SDS and extracted lipids in acetone or in n-butanol. The lipid free protein was solubilized again in SDS. This protein fraction with an S value of 2.3 consisted mainly of non-haem protein but included some cytochrome f and b_6 in approximately equimolar amounts.

Tae *et al.* (1968) extracted spinach chloroplast lamellae by repeated slow acetone extraction. The protein obtained was heterogeneous on gel electrophoresis with an S value of 2.2. Lokshin and Burris (1966) isolated lamellar proteins from chard and corn. They solubilized the sonicated lamellae with n-butanol at 25°C. Criddle and Park (1964) isolated a protein fraction from the spinach lamellae by ammonium sulphate precipitation and called it chloroplast structural protein. The chloroplast structural proteins obtained from wheat and bean were heterogeneous on gel electrophoresis (Mani and Zalik, 1970).

Many detergents like digitonin, Triton X-100, SDS and SDBS have been used for the fragmentation of chloroplast lamellae. Anderson and Boardman (1966) obtained two classes of particles on the incubation of the lamellae with digitonin. On the basis of the ratio of chlorophyll a/b and the photochemical activity of the particles they suggested that these particles were representative of PSI and PSII activity. On the other hand, Triton X-100 treated lamellae gave light particles possessing PSI activity (Vernon *et al.*, 1966).

Thornber *et al.* (1967a, 1967b) obtained two pigment protein bands on polyacrylamide gel electrophoresis of sodium dodecyl benzene sulphate solubilized lamellae *Beta vulgaris* chloroplasts and reported them as chlorophyll-protein complexes, both of which were photochemically inactive. Machold (1971) noted 17 bands from SDS solubilized lamellae of *Phaseolus vulgaris* and reported that two of them were chlorophyll-protein complexes like those of Thornber *et al.*. In *Antirrhinum majus* 15 bands were reported by Herrmann and Meister (1972) who claimed that 7 colored bands were chlorophyll-protein complexes which were visible only during the first 90 min of electrophoresis and then vanished gradually.

So far little is known about the assembly of membrane specific proteins formed during greening in higher plants. However, Eytan and Ohad (1970) and Hoober (1970) have studied the site of synthesis of membrane polypeptides during biogenesis of chloroplast lamellae using antibiotic inhibitors and a y-1 mutant of *Chlamydomonas reinhardi*. The algae failed to synthesize chlorophyll and disc membranes in the dark. On addition of chloramphenicol and cycloheximide the formation of membranes was inhibited which suggested that chloroplast and cytoplasmic ribosomes were involved in protein synthesis of the membranes of the chloroplast. This has been previously observed in other plants by a number of workers (Smillie *et al.*, 1968; Margulies, 1971; Ireland and Bradbeer, 1971).

Hoober (1972) found that synthesis of a main polypeptide of the thylakoid membrane occurred in the cytoplasm in soluble form and its synthesis was coupled with the synthesis of chlorophyll. Eytan and Ohad (1970) reported that the proteins produced on cytoplasmic ribosomes assembled together with lipids and chlorophyll forming a non-active membrane. It was found that the proteins formed on chloroplast ribosomes which they termed activation proteins were essential for the formation of a photosynthetically active membrane. The experiments suggested that synthesis of these proteins does not require light, however, synthesis of chlorophyll controlled the synthesis of cytoplasmic proteins at the transcriptional level which in turn controlled synthesis of activation proteins (Eytan and Ohad, 1972). Similar conclusions were reached independently by Hoober and Stegeman (1973), that a precursor of chlorophyll regulated transcription of mRNA for polypeptide synthesized in the cytoplasmic ribosomes and its regulation is mediated by a protein synthesized within the chloroplast.

Tubule-like structures of the chloroplast and microtubules

Recently it has been shown that microtubules occur in both plant and animal cells (Tilney, 1968; Newcomb, 1969). Microtubules are unbranched tubular structures of indefinite length. In the plant cell they lie just beneath the cell membrane, parallel to cellulose microfibrils and these microtubules are morphologically similar to the microtubules which constitute the fibers of mitotic spindles (Ledbetter and Porter, 1963). In animal cells they compose the fibers of the mitotic spindle, tubular structures in centriole and the basal bodies. The 9+2 array of axonemal filaments of cilia and flagella of motile plant and animal cells is made up of microtubules. The exact nature of their function is now known. It is believed that they participate in the motile system of cilia and flagella, perform a cytoskeletal role in many cells and perhaps help in movement of cytoplasmic material.

These subcellular structures are seen in the tissue only when fixed in glutaraldehyde-OsO_4 but are usually destroyed if fixed in OsO_4 alone or in $KMnO_4$.

The diameter of a tubule ranges from 180-300 Å, the average value being about 240 Å (Newcomb, 1969). In cross section they show an electron lucent core about 100 Å in diameter bounded by an electron opaque ring about 70 Å thick. The nature of the material present inside the core of the tubule is not known. The wall of plant microtubules is probably composed of 13 filamentous subunits (Ledbetter and Porter, 1963). Recent studies on isolated microtubules suggested that the filaments of the wall were composed of a series of globular subunits. Biochemical work on cilia, protozoan flagella and sperm tail provided evidence that their microtubules were made up of actin-like proteins called "tubulin". Its amino acid composition and ability to associate with nucleotide resembled that of actin from muscle (Stephen, 1967). Cholchicine and related compounds were highly active in disrupting the mitotic spindles. Borisy and Taylor (1967) proposed that cholchicine acted by binding to subunit protein and preventing its polymerization into microtubules.

Microtubule-like, slender, unbranched structures have been frequently seen in the stroma of developing chloroplasts of *Hordeum vulgare* (Sprey, 1968; Henningsen and Boynton, 1970). They were seen between the thylakoids, rarely attached to the lamellae. The size of these tubules was smaller than the microtubules present in the cytoplasm of plant and animal cells. In cross section these structures revealed an outer electron dense core 110 Å surrounding an electron transparent ring of 30 Å. In higher magnifications the tubules appeared hexagonally arranged in crystalline arrays of 10 to 80 units (Sprey, 1968). Henningsen and Boynton (1970) related these structures to the stroma centers found in *Triticum aestivum* and *Avena sativa* chloroplasts. Sprey (1968) had suggested that they participate in the formation of thylakoids.

Endocytosis.

Amoeboid protozoa and leucocytes have the ability to engulf large pieces of particulate matter. When an *amoeba* comes into contact with food particles it forms doughnut-shaped projections of the cytoplasm around the food particles thus enclosing the latter (Jennings, 1904). The process was named phagocytosis by Metchnikoff in 1883. Another closely related process, in which cells ingest large amounts of particle free fluid was noted by Edward (1925) in *amoeba*. Later in 1931 Lewis observed it in macrophages and other cultured tissue and he named it pinocytosis. Both processes are encompassed by the term endocytosis which provides a means of active ingestion of molecules to which cell membranes are impermeable. The occurrence of the process has been reported in widely different tissue throughout the plant and animal kingdoms and evidence for this has been reviewed by Holter (1959) and Rustad (1964). Several workers have reported entry of macromolecules into plant cells. In the electron microscope studies of plant cells, bays and infolding of plasma membranes have been observed by several workers, and Whaley *et al.* (1964) have considered the possibility that a process akin to pinocytosis and phagocytosis may take place in the plant cells. Entry of labelled histone, lysozymes and poly-L-lysine into barley root tips was reported by Drew *et al.* (1970). Wheeler *et al.* (1972) have reported pinocytotic uptake of uranyl acetate in oat root tips. Good evidence for the occurrence of the process of pinocytosis has been provided by the study of isolated protoplasts. Mayo and Cocking (1969) reported that isolated tomato fruit protoplasts take up tobacco mosaic virus and polystyrene latex particles by means of pinocytosis (Cocking, 1970). On the basis of generally accepted terminology it seems that the process should have been called either phagocytosis or endocytosis.

MATERIALS AND METHODS

Plant Material.

The chloroplyll deficient mutant used in this study is a virescens type of viridis mutant of *Hordeum vulgare* c.v. Gateway. In our laboratory it has been compared with the parent Gateway on a biochemical, genetic, physiological and ultrastructural basis (Maclachlan and Zalik, 1963; Miller and Zalik, 1965; Sane and Zalik, 1970; Stephansen and Zalik, 1971). It has been confirmed that the chlorophyll deficiency is governed by a single recessive nuclear gene (Walker *et al.*, 1963; Stephansen and Zalik, 1971). In all the studies being reported Gateway barley and its mutant were grown simultaneously in the same growth cabinet.

Growing Seedlings.

To study the development of chloroplasts from etioplasts, seeds were soaked in water for 3 to 4 hr and spread on vermiculite in plastic trays (25 seeds/tray) which were covered with wet filter paper. The trays were kept in a dark growth cabinet at 23°C for 7 to 10 days and were watered daily. After 7 or 10 days the etiolated seedlings which had grown to a height of 13 cm or 16 cm respectively were selected under a green safelight for exposure to white light and the others were discarded. The trays were transferred to a growth cabinet and illuminated for varying periods. In some cases the light period was followed by a further dark period. The duration of illumination was based upon results from preliminary experiments.

For comparison of the chloroplast tubules with cytoplasmic microtubules a cold treatment experiment similar to that of Benke and Forer (1967) was performed. Ten-day dark grown seedlings were illuminated for 6 hr followed by a 3 hr dark period. Intact seedlings or excised leaves in 0.01 M phosphate buffer, pH 7.3, were placed in a cold room at 4° ± 2°C. Leaf samples from these were fixed after 1, 2 and 3 hr. Some seedlings which were in the cold room for 3 hr were placed at room temperature for 30 min and samples of these were also fixed for EM studies. For biochemical investigation, seeds of the normal and mutant barley were planted in 20 x 25 cm plastic trays containing wet vermiculite. The trays were kept in the growth cabinet at 22° ± 1°C under continuous illumination at an average intensity of 1400 ft-c (range 1200-1600 ft-c) and relative humidity of about 55%. They were harvested after 4, 6 and 8 days. Prior to harvesting the trays were left overnight in a darkroom to deplete the chloroplasts of starch.

From each tray pieces of 5 leaves were fixed in glutaraldehyde-OsO_4 for EM. Fifteen to 20 leaves were freeze-dried for pigment determination and 10 g of leaves were used for the isolation of lamellar proteins. Identical determinations were conducted on plants grown under 600 ft-c (range 550-650 ft-c) at 20° ± 1°C. From the latter soluble leaf proteins were also isolated.

Chemicals.

The suppliers of various chemicals used in the study were: Araldite, dodecenyl succinic anhydride (DDSA), dimethyl amino methyl phenol (DMP30) and lead citrate from Ladd Research Industries (Burlington, Vermont, USA); OsO_4 from Johnson Mathey Chemicals Limited (Hatton Garden, London, England); uranyl acetate from Mallinckrodt Chemical (St. Louis, Missouri, USA); acrylamide, N,N^1-methylene *bis*-acrylamide, N,N,N^1,N^1-tetramethyl ethylenediamine, propylene dioxide and 2-mercaptoethanol from Eastman Organic Chemicals (Rochester, N.Y., USA). Ammonium persulphate, sucrose, boric acid, trichloroacetic acid, sodium hydroxide, glycine, glutaraldehyde (Biological grade), $MgCl_2$ from Fisher Chemicals (Edmonton, Alberta, Canada); sodium phosphate, calcium carbonate from J.T. Baker Chemical Co. (Phillipsburg, N.J.); ficoll, Dextran T-250 from Pharmacia (Uppsala, Sweden); tris-(hydroxylmethyl) aminomethane from Raylo Chemical Limited (Edmonton, Alberta, Canada); coomassie blue from Consolidated Laboratories Ltd. (Canada) and amido black from Allied Chemical (Morristown, N.J.).

Preparation of Material for EM Studies.

Sections, approximately 2 mm sq., were cut with an acetone cleaned blade from either side of the midrib 1 cm from the leaf apex under buffer (0.01 M sodium phosphate buffer pH 7.1-7.3). Each piece was fixed separately in a vial containing 3% glutaraldehyde in the buffer for 3 hr at room temperature. After four changes of the buffer during two hr, post fixation was carried out for two hr in 2% OsO_4. Following quick rinsing in distilled water the tissue was dehydrated in a graded ethanol series. The dehydrated tissue was then embedded in an araldite resin mixture (Luft, 1961) and four blocks from each treatment were sectioned with a glass or a diamond knife on a Sorvall MT-2 microtome. Sections were stained with 2% uranyl acetate followed by 0.2% lead citrate (Reynolds, 1963) and were examined under a Philips EM 100 or 200 electron microscope.

Pigment Determination.

Freeze-dried leaf samples of normal (20 mg) and mutant barley (40 mg) were weighed and ground with a total of 25 ml of 80% acetone in a glass mortar containing a small amount of acid washed sand and $CaCO_3$. Thorough repeated grinding was required for complete extraction of the pigments. The pigment extract was cleared by centrifugation at 10,000 x *g* for 15 min. It was decanted and made to a total volume of 25 ml in a volumetric flask. Its absorbance was determined at 440, 645 and 663 nm in a Beckman DK-1 Spectrophotometer and the concentrations of chl a, chl b and carotenoids were calculated (Maclachlan and Zalik, 1963).

Extraction of Soluble Leaf Proteins.

The frozen leaves were ground in a chilled mortar at

4°C using tris glycine buffer (3.6 mM glycine and 0.5 mM tris) pH 8.3 (1:1 w/v). The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 90,000 x *g* for 2 hr. An aliquot of the supernatant was used for protein estimation by the Lowry method (Lowry *et al.*, 1951). The remainder was frozen immediately and after protein estimation a sample was taken from it for electrophoresis the same day.

Isolation of Chloroplast Soluble and Lamellar Proteins.

The medium for chloroplast isolation, I, was 0.01 M phosphate buffer, pH 7.8, containing 2.5% ficoll, 5% Dextran T-250, 0.25 M sucrose, 10 mM 2-mercaptoethanol and 2 mM Mg. Lamellae were prepared by extracting the soluble proteins with 0.16 M sodium borate-HCl buffer, pH 8.9 (buffer II) and the lamellar proteins were then extracted with the same buffer containing 0.5% SDS (buffer III). The ratio of fresh leaves to isolation medium (I) was 1:5 (w/v). The leaves were ground in a mortar and squeezed through 16 layers of cheesecloth. The procedure followed in isolating the lamellar proteins from the leaf filtrates is diagrammed in Figure 3. All operations were at 4°C. The amount of lamellar protein was estimated on an aliquot of the final supernatant. The proteins were precipitated by 10% cold trichloroacetic acid and the pigments were extracted with acetone. The pigment free residue was solubilized in 1N NaOH and the amount of protein was estimated. The supernatants obtained by centrifuging the

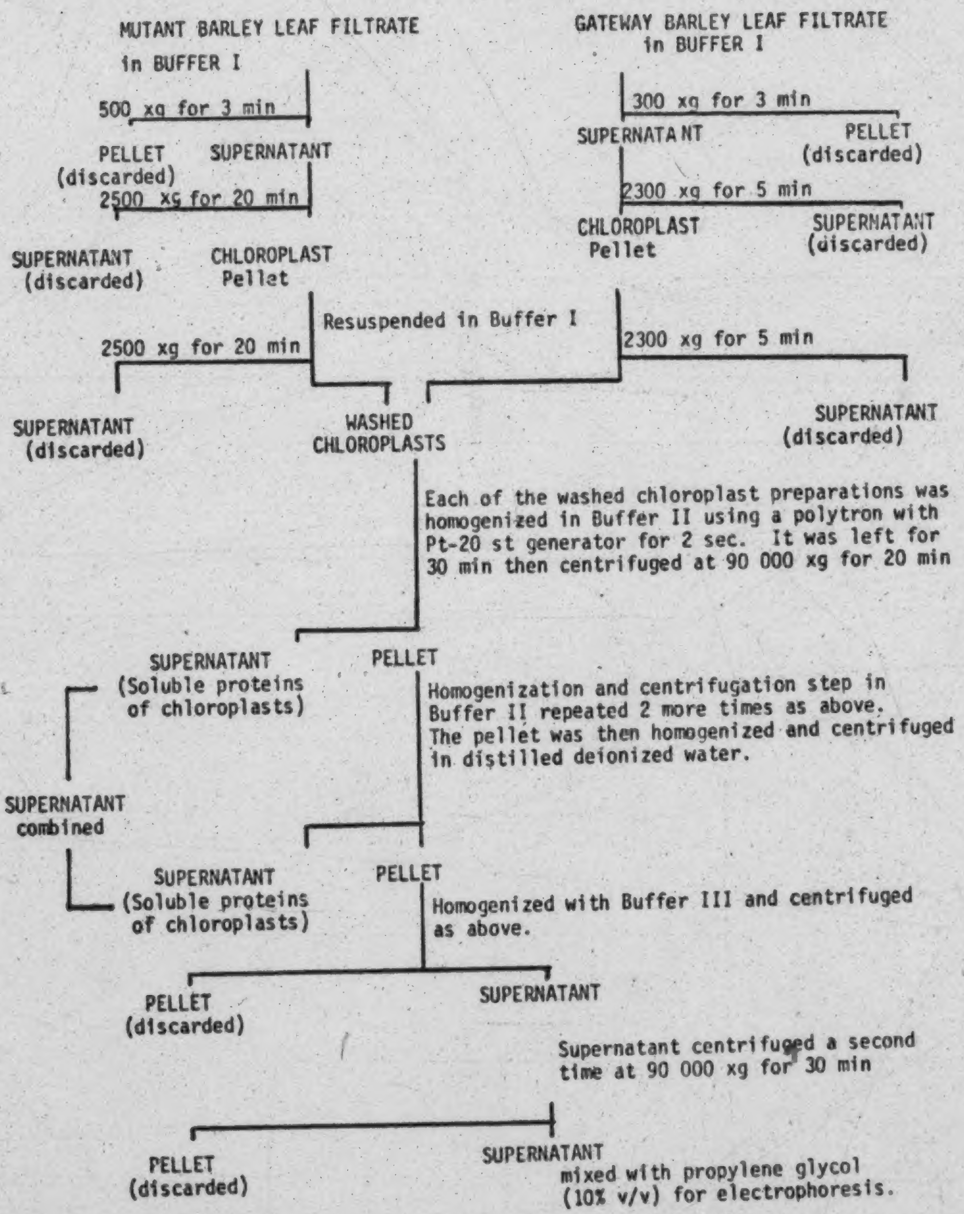

MUTANT BARLEY LEAF FILTRATE
in BUFFER I
500 xg for 3 min
PELLET (discarded)
SUPERNATANT
2500 xg for 20 min
SUPERNATANT (discarded)
CHLOROPLAST Pellet
GATEWAY BARLEY LEAF FILTRATE
in BUFFER I
300 xg for 3 min
SUPERNATANT
PELLET (discarded)
2300 xg for 5 min
CHLOROPLAST Pellet
SUPERNATANT (discarded)
Resuspended in Buffer I
2500 xg for 20 min
2300 xg for 5 min
SUPERNATANT (discarded)
WASHED CHLOROPLASTS
SUPERNATANT (discarded)
Each of the washed chloroplast preparations was homogenized in Buffer II using a polytron with Pt-20 st generator for 2 sec. It was left for 30 min then centrifuged at 90 000 xg for 20 min
SUPERNATANT (Soluble proteins of chloroplasts)
PELLET
Homogenization and centrifugation step in Buffer II repeated 2 more times as above. The pellet was then homogenized and centrifuged in distilled deionized water.
SUPERNATANT combined
SUPERNATANT (Soluble proteins of chloroplasts)
PELLET
Homogenized with Buffer III and centrifuged as above.
PELLET (discarded)
SUPERNATANT
Supernatant centrifuged a second time at 90 000 xg for 30 min
PELLET (discarded)
SUPERNATANT
mixed with propylene glycol (10% v/v) for electrophoresis.

FIGURE 3. Procedure used in isolating chloroplast lamellar proteins. 10 g of leaves ground with 50 ml buffer I in a mortar and pestle. Brei strained through 16 layers of cheesecloth.

chloroplasts in buffer II containing soluble chloroplast proteins were combined, dialyzed overnight against water and freeze-dried. Samples were dissolved in tris-glycine buffer, pH 8.3, for protein estimation and electrophoresis.

Electrophoresis.

The proteins were electrophoresed as described by Davis (1964). For separation of soluble leaf proteins, gels containing 9.5% acrylamide and 0.28% *bis*-acrylamide were used at pH 8.3. Distilled deionized water was used throughout. The stock solutions used to prepare gels were: (A) acrylamide 36 g and *bis*-acrylamide 1.12 g, (B) tris 36.6 g, TEMED 0.25 ml, 48 ml of 1N HCl, (C) riboflavin 5 mg. Each stock solution which was made up to 100 ml with water was filtered through Whatman No. 2 filter paper.

Gels were prepared in 6.0 x 0.5 cm glass tubes cleaned with chromic acid and coated with Kodak photo-flo solution (1 part in 200 ml water). The lower end of the tubes was sealed with parafilm and the tubes were positioned vertically with a rubber band on a flat ruler which was fixed horizontally to a stand by a mechanical clamp. The gel solution was made by mixing A, B, C and water in ratio of 2:1:1:4. It was poured carefully in the glass tubes with a Pasteur pipet. A buffer layer of 0.5 cm was placed on the top of the gel solution. The gels were photopolymerised with a fluorescent tube which was positioned about 1 inch from the gel tube. After polymerization the tubes were kept at 4°C. Electrophoresis was carried out in a pre-cooled tris-glycine buffer (3.6 mM glycine and 0.5 mM tris) pH 8.3 at 4°C. A drop of 0.01% bromophenol blue in water as marker was added to the upper buffer reservoir. A sample containing 75 to 100 μg of protein and 3% sucrose was applied to each gel. The runs were started at 2 mA/tube for 20 min then the current was increased to 3 mA/tube. The total time for electrophoresis was approximately 2 hr. The gels were stained overnight in a 1% solution of amido black in 7% acetic acid and they were destained electrophoretically in 5% acetic acid perpendicular to the direction of separation.

For lamellar proteins, gels were prepared in 12.0 x 0.5 cm glass tubes. The stock solutions used to prepare the gels were: (A) acrylamide 36 g and *bis*-acrylamide 2 g, (B) acrylamide 10 g and *bis*-acrylamide 2.5 g, (C) tris 36.6 g, TEMED 0.25 ml, in 48 ml of 1N HCl, (D) ammonium persulphate 0.14 g. Each stock solution was made up to 100 ml with water. The lower small pore gel solution was made by mixing A, C, D and water in the ratio 2:1:4:1. This was poured first in the tubes to a depth of 10 cm and a 0.5 cm layer of water was poured on the top of it. The gels were chemically polymerized at room temperature for 30-45 min. After polymerization of small pore gel the water layer was absorbed by a piece of filter paper. Then the large pore gel solution was made by mixing B, C, D and water in ratio, 2:1:4:1 and this was layered to a depth of

1.5 cm on the polymerized small pore gel followed by 0.5 cm layer of water.

Extract containing 50 to 100 μg protein was layered on the large pore gel under buffer. Electrophoresis was performed at 24°±2°C in 0.05 M sodium borate-HCl buffer, pH 8.3, containing 0.01% SDS. Runs were made at 3 mA/tube for 4 hr and the gels were stained for 24 hr with coomassie brilliant blue, 0.1% in acetic acid:methanol:water, 1:1:8, followed by destaining with the same solution without dye for at least 2 days.

RESULTS AND DISCUSSION

I. Chloroplast Development in Gateway Barley and Its Mutant

Results

Greening of 7-day-old etiolated barley seedlings.

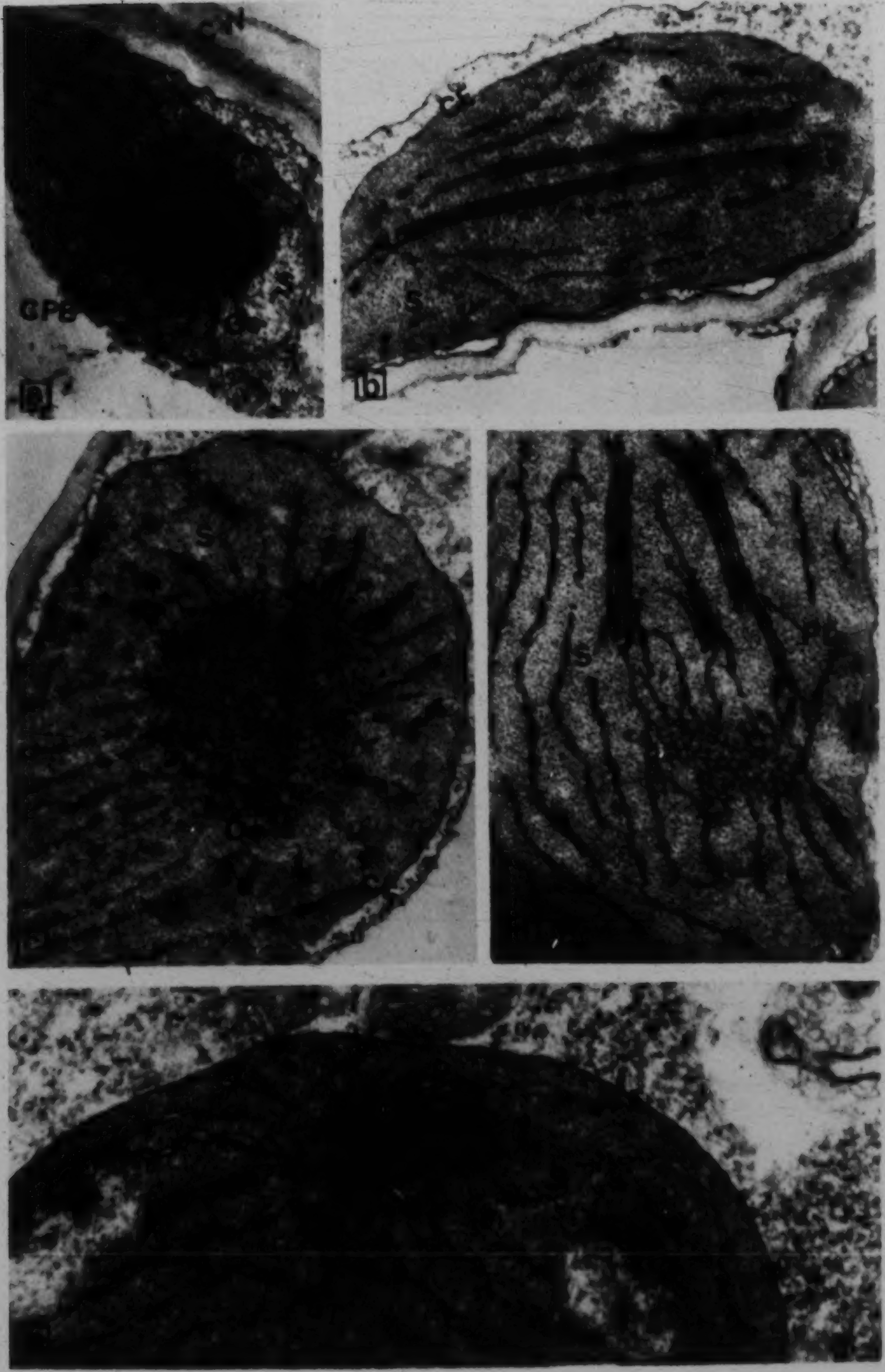
The 7-day-old dark-grown seedlings of Gateway barley contained plastids of the type shown in Fig. 4a and b. Crystalline prolamellar bodies were present in most of these plastids. A number of primary layers (PL) were radiating out of the crystalline prolamellar bodies. This agrees with the observation of von Wettstein (1958) that protrusion of the vesicles from the prolamellar body and the formation of primary layers occurs in the etioplasts of barley. Some osmophilic granules were interspersed in the prolamellar body. Plastids which did not have a prolamellar body were smaller, less developed and had a few primary layers (Fig. 4b).

On exposure to varying periods of light of 1400 ft-c a marked series of structural changes were observed in the prolamellar bodies of the etioplasts. Plastids of etiolated leaves exposed to 5, 10, 15, 20, 30 and 45 min and up to 2 days of illumination were examined. There was a gradual decrease in the percentage of plastids with prolamellar bodies with extension of the period of illumination. The

FIGURE 4. Plastids from Gateway barley seedlings grown in the dark for 7 days and then exposed to light for various periods of time.

Etiolated (a) A crystalline prolamellar body and a number of lamellae radiating from it. X 21,000. (b) A plastid with a few primary layers. X 30,000. Exposed to light for 5 min. (c) A prolamellar body showing an aggregated mass of membrane tubules and osmophilic granules (Os). X 22,100. Exposed to light for 30 min. (d) Dispersal of membrane tubules of a prolamellar body and the formation of primary and secondary layers in the stroma are noted. X 22,000. (e) Most of the material of the prolamellar body is dispersed and in its place numberous osmophilic granules are seen. The arrow indicates tubes extending from the prolamellar body. X 32,500.

The light intensity used for this and subsequent figures unless indicated otherwise, was 1400 ft-c. Fixation for this and the succeeding figures was with glutaraldehyde and OsO_4. Any exception to this will be indicated.


CW
GPB
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a
CE
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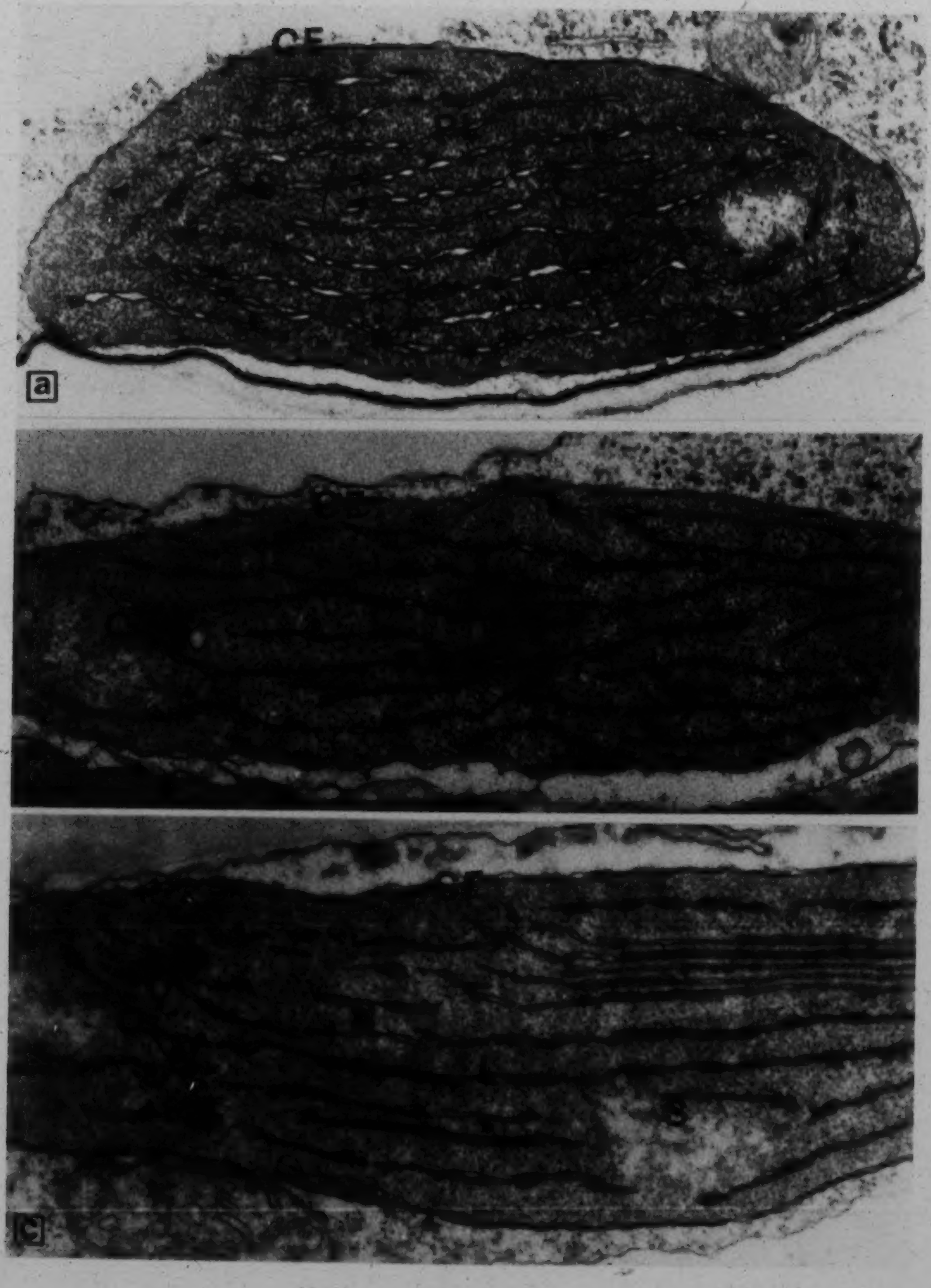
b
S
S

transformation of the prolamellar bodies and dispersal of their tubular material was completed within an hour. First the crystalline order of the prolamellar body disappeared and an aggregated mass of tubules or vesicles appeared in its place (Fig. 4c and e). Within half an hour the tube material of the prolamellar body started to disperse. Fig. 4e shows tubules extending out of the prolamellar body. A number of osmophilic granules were concentrated at the place of the prolamellar body, but sections of many plastids did not show osmophilic granules (Fig. 4d). Because these granules initially occurred only at the site of the prolamellar bodies their presence may have been related to the portion of the plastid that was examined.

Dispersal of tubule material occurred in an ordered manner. As the tubules started to disperse they joined end to end and aligned themselves in rows, which were generally parallel. In some instances during the dispersal process secondary layers were formed simultaneously along primary layers. The dispersal process was completed within 1 hr of illumination and the plastids showed a number of primary layers (Fig. 5a). In these sections no plastids showed prolamellar bodies. The arrows (Fig. 5a) indicate the places of overlapping and adjoining of the lamellae. Within 3 to 6 hr multiplication of the primary layers of the plastids was initiated in different portions to form secondary layers (Fig. 5b and c).

After illumination for 9 hr the plastids showed

FIGURE 5. Plastids from Gateway barley seedlings grown in the dark for 7 days and then exposed to light.
(a) 1 hr of light. Primary layers are seen and the arrow indicates the places of overlapping and adjoining of lamellae. X 33,800.
(b) and (c) 3 and 6 hr light respectively. The vesicular space inside the layers is reduced and multiplication at different portions of the lamellae is observed. (b) X 39,000. (c) X 43,900.


CE
a
S
c

numerous grana, each composed of two thylakoids (Fig. 6a). The number of thylakoids in the grana increased with the time of illumination. Fig. 6b shows plastids of seedlings exposed for 12 hr containing grana and stroma lamellae. From an examination of the various areas of the lamellae where thylakoid stacking was in progress (arrows) it appears that multiplication resulted by the process of protrusion and invagination of the lamellae (Menke, 1962, 1964; Wehrmeyer and Röbbelen, 1965).

The development of chloroplasts from etioplasts was completed in a period of 2 days by which time the chloroplasts had characteristic features such as grana lamellae, stroma lamellae and starch grains. They appeared like fully developed chloroplasts (Fig. 15).

During exposure to light the development of plastids of the mutant barley was considerably slower than those of the normal barley. Plastids from the dark grown seedlings of the mutant were smaller in size and lacked interior organization (Fig. 7). On the basis of their morphological appearance four types of plastids were present in the mutant: 1) plastids with vesicles (Fig. 7a); 2) undifferentiated plastids with fewer vesicles (Fig. 7b and c); 3) plastids with an inconspicuous prolamellar body containing small osmophilic granules and a few primary layers radiating out of the prolamellar body (Fig. 7d); 4) plastids with a few thylakoids. The stroma of all the plastids was very dense.

FIGURE 6. Plastids from Gateway barley seedlings grown in the dark for 7 days and then exposed to light.

(a) 9 hr light. A number of grana composed of two thylakoids. X 42,300.

(b) 12 hr light. A plastid with grana and stroma lamellae. Arrows indicate stacking of thylakoids. X 51,800.

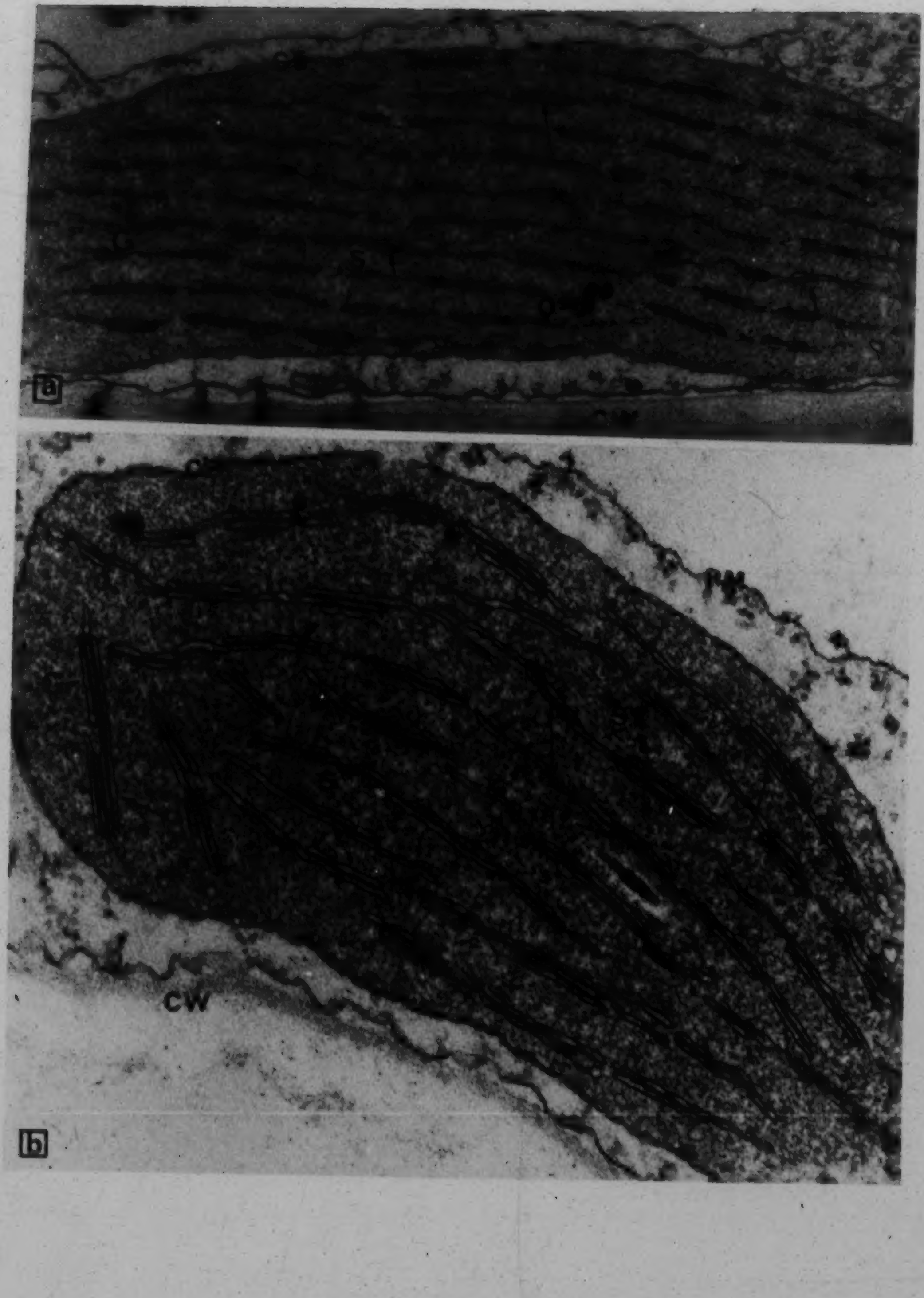

S
a
C
PM
CW
b

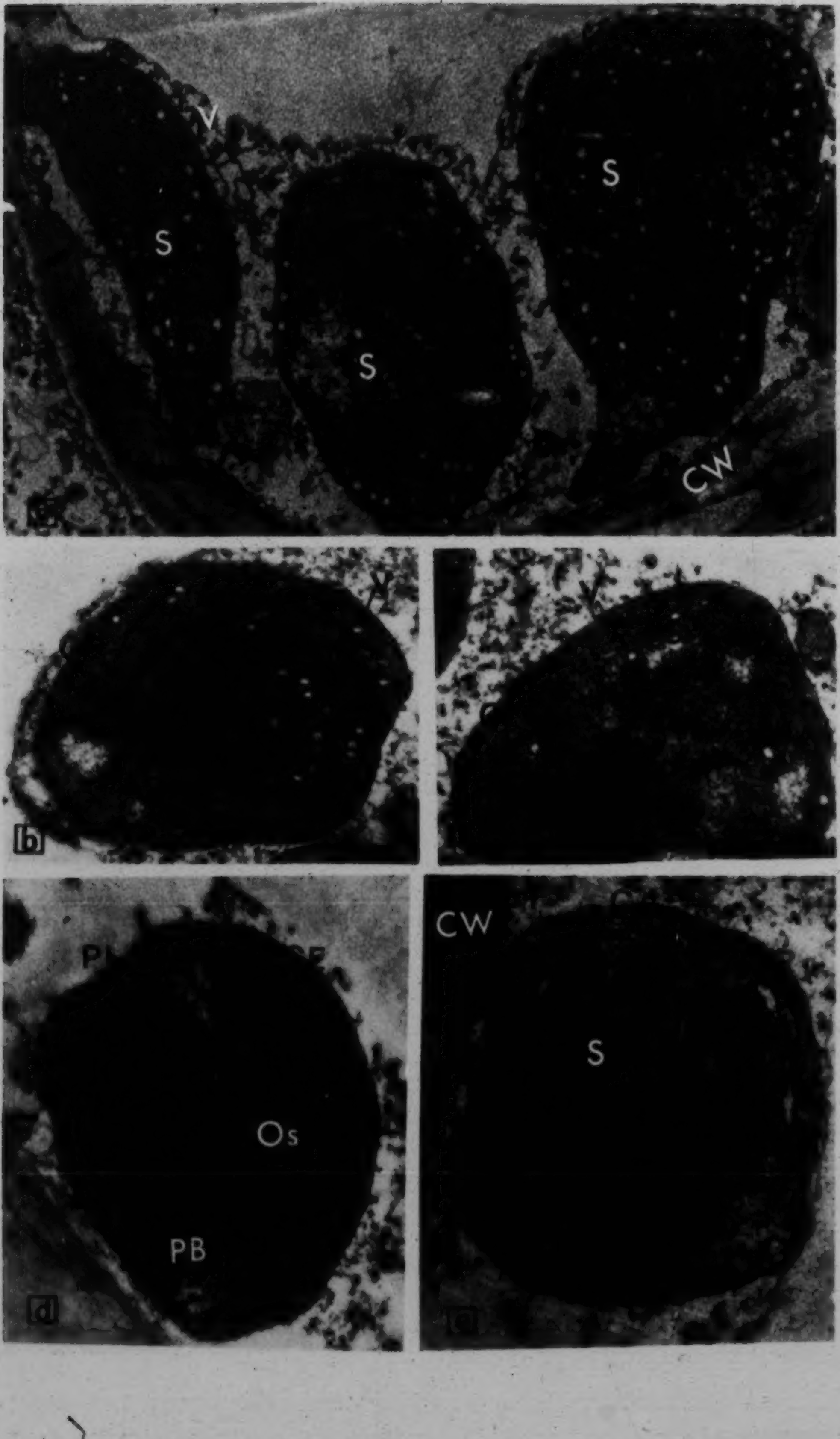
FIGURE 7. Types of plastids present in 7-day-old dark grown seedlings of mutant barley.

(a) Plastids showing numerous vesicles (V). X 22,300.

(b) and (c) Undifferentiated plastids with fewer vesicles. X 21,000.

(d) Plastid with inconspicuous prolamellar body and a number of primary layers extending out of the prolamellar body. X 20,000.

(e) Plastid with a few thylakoids. X 45,600.


V
S
S
S
S
CW
V
b
V
PL
Os
PB
d
CW
S

In seedlings treated for 1 hr in light no major changes were observed in the structure of the plastids. Fig. 8a and b show typical plastids observed at this stage. The plastids showed vesicles with a few membrane tubules (Fig. 8a). Not all the plastids had vesicles, in fact, within the same cell or in adjacent mesophyll cells some plastids appeared like normal developing plastids except for their smaller size. Fig. 8b shows a plastid with dispersed membrane tubules.

Fig. 8c shows part of a plastid from seedlings illuminated for 3 hr. The size and number of vesicles increased in these plastids. A number of vesicles were seen joining together (arrow), and this process continued for another 3 hr (Fig. 8d). There appeared to be very little increase in the amount of lamellar material during this period. For seedlings which had been exposed for 12 to 24 hr the vesicles, which were dispersed in the plastid throughout the stroma, changed their shape (Fig. 8e). The arrow indicates the transformation of a vesicle into a tube. The vesicles may flatten and become more or less like thylakoid discs. These thylakoid discs stack together either side by side and initiate the formation of grana, or join end to end to form primary layers.

The process of thylakoid formation from the vesicles and grana formation from these thylakoids continued until all the vesicles were transformed. After 2 to 4 days of illumination plastids containing isolated grana were

FIGURE 8. Developing plastids from 7-day-old dark grown seedlings of mutant barley illuminated for various periods.

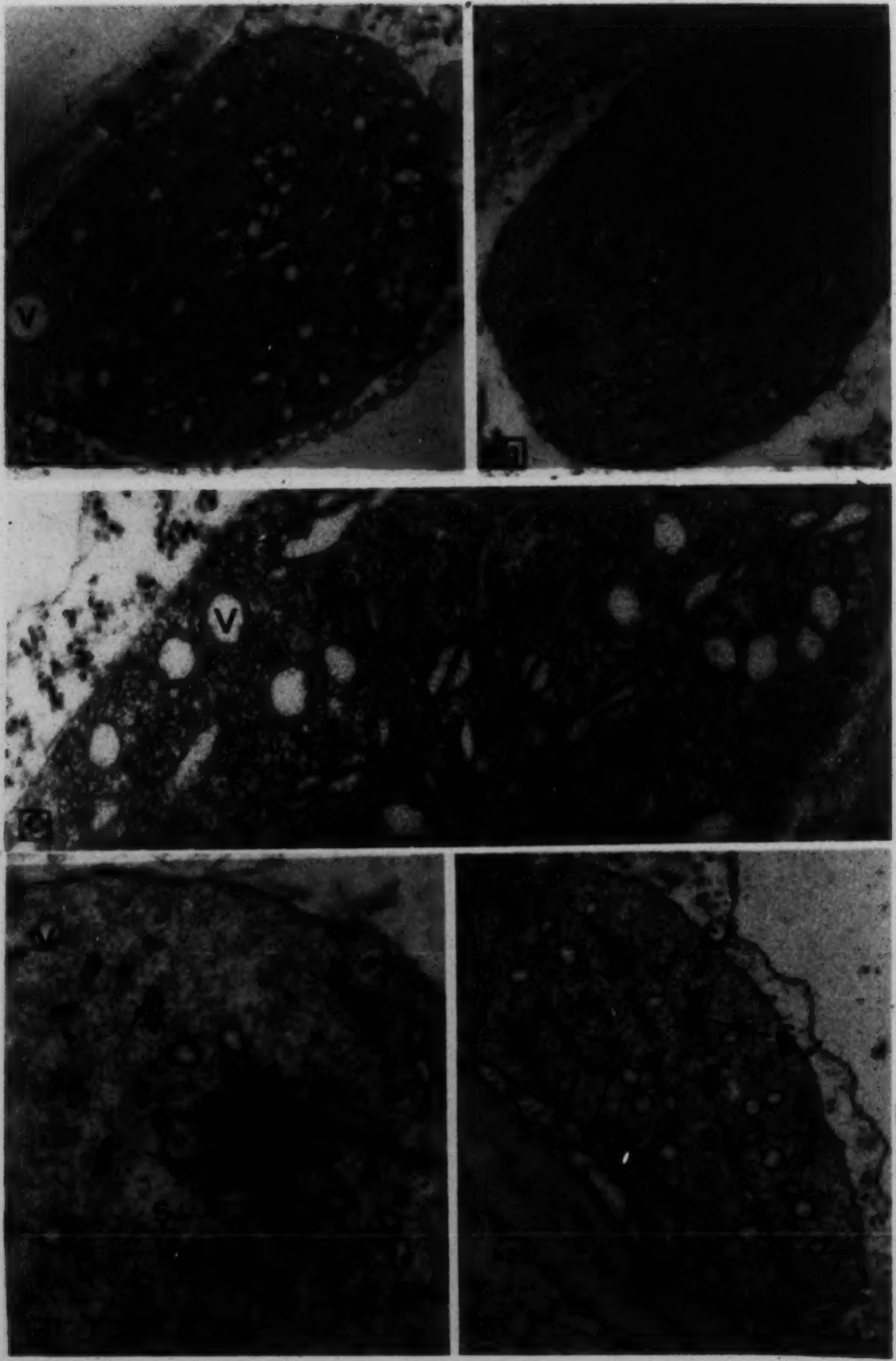
One hour light. (a) Plastid with many vesicles. X 32,500. (b) Plastid similar to a normal barley plastid, containing a few thylakoids. X 33,000.

Three hours light. (c) Part of a plastid showing joining of some of the vesicles (arrow). X 67,200.

Six hours light. (d) Plastid showing dispersed vesicles (V) and tubules (T). Long arrow shows joining of the two tubules side by side. Both the vesicles and/or tubules are seen aligned end to end (short arrows). X 44,700.

Twelve hours light. (e) The vesicle has been transformed into small thylakoids (arrow).

Grana (G) formed by joining of thylakoids. X 76,500.


V
b
V
c
V
S

observed (Fig. 9a). The process of vesicle transformation and grana formation occurred simultaneously. The arrow indicates the flattening of a vesicle which is forming an end granal thylakoid. Many plastids at this age still contained a few vesicles. Stroma lamellae appeared after 6 days of illumination. A representative plastid of this age is shown in Fig. 10a. By 4 to 6 days of illumination, the plastids had increased in size (Fig. 10b) but they were still smaller than those in normal barley.

Abnormally large grana formed by stacking of 30 to 50 long lamellae were observed in the mutant during grana formation. Fig. 9b shows a plastid from a seedling illuminated for 6 days containing a few small grana and two large grana surrounding a starch grain. Such grana were also observed in the plastids of 2- and 4-day illuminated seedlings.

The amount of lamellar material in the plastids of mutant barley increased gradually with illumination throughout the study. The 10-day illuminated seedlings had plastids of the type shown in Fig. 10b. Grana in face view and in side view were seen in the same plastid. Therefore thylakoid stacks can be arranged in different directions to each other. Stroma lamellae were observed running in different directions to connect grana lamellae together.

In 14-day illuminated seedlings the lamellar system of the chloroplast (Fig. 10c) occupied most of the stroma and the number of osmophilic granules had increased.

FIGURE 9. Developing plastids from 7-day-old dark grown seedlings of mutant barley illuminated for various periods of time.

Two days of illumination. (a) Plastid with isolated granal stacks not connected by stroma lamellae. The arrows indicate flattening of a vesicle which is forming an end granal thylakoid. X 38,400.

Six days illumination. (b) Stacking of 30-50 abnormally long lamellae to form large grana which surround the starch grain (St). X 44,800.

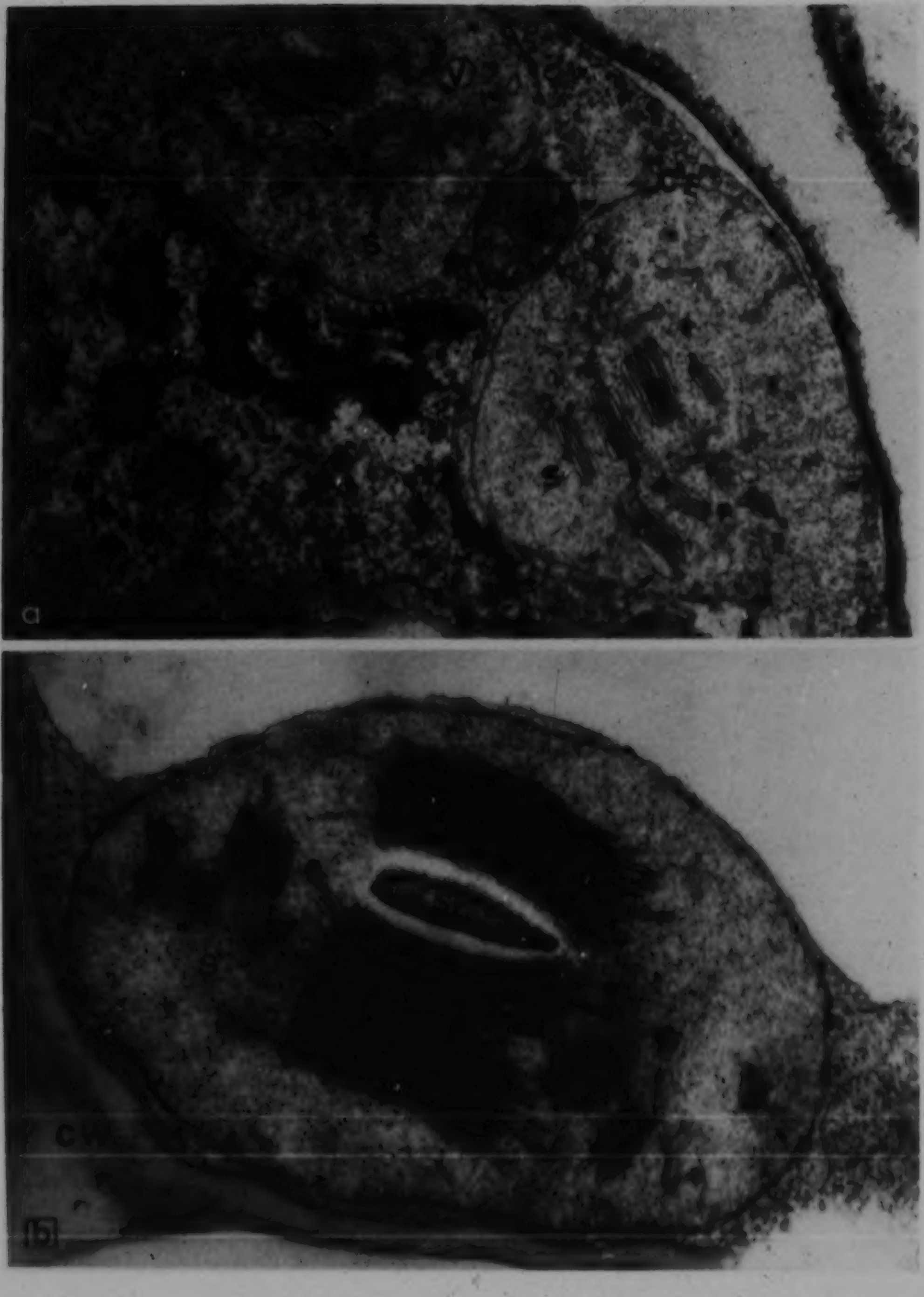

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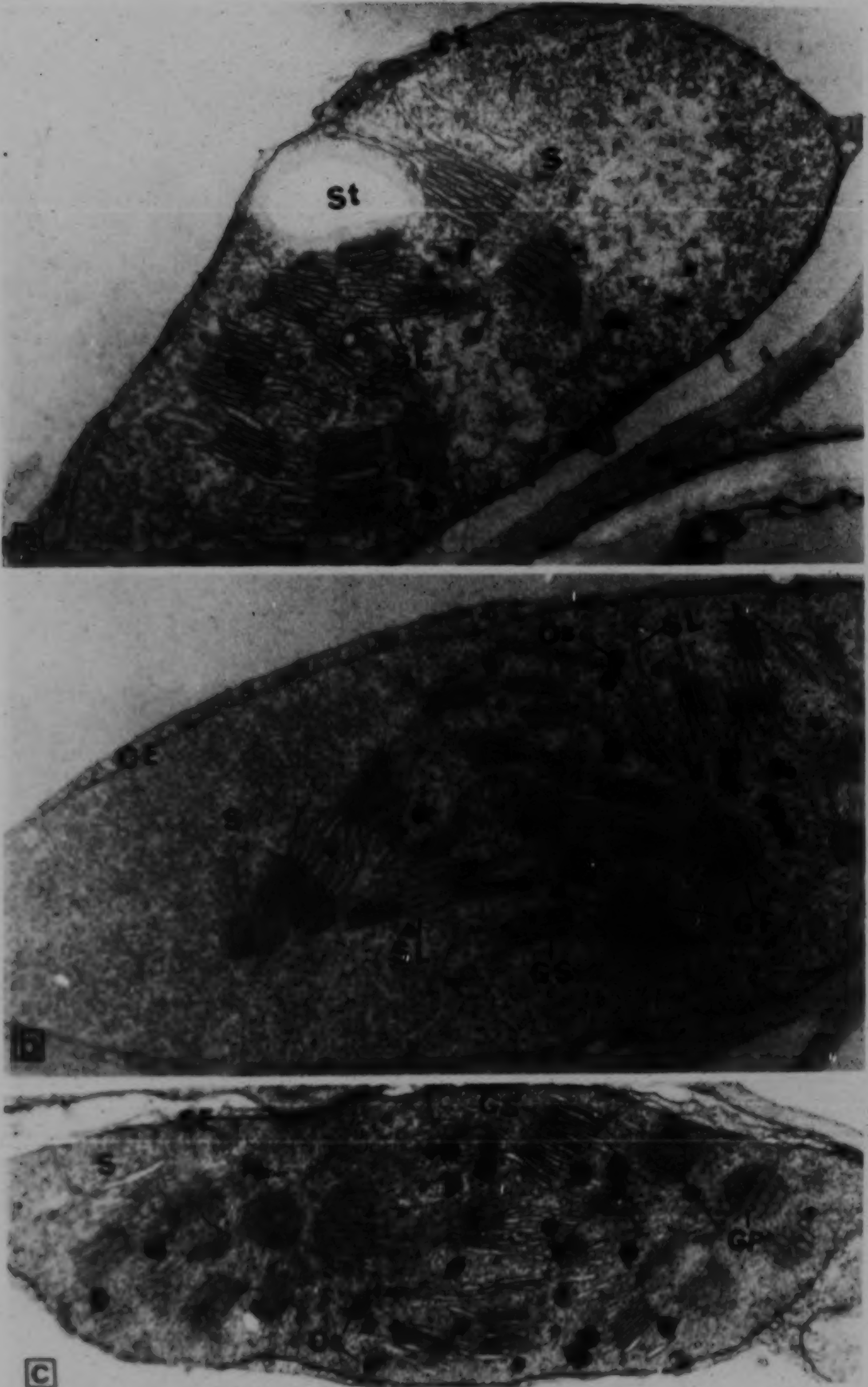
FIGURE 10. Plastids from 7-day-old etiolated mutant barley seedlings exposed to light for 6, 10, and 14 days.

Six days illumination. (a) In this plastid the grana are arranged in a plane different from that usually observed in normal chloroplasts. A few stroma lamellae (SL) are seen joining grana (G) together. X 37,500.

Ten days illumination. (b) A plastid with grana in face view (GF) and side view (GS) and interconnecting stroma lamellae. The grana stacks are not all aligned in the same plane. X 42,300.

Fourteen days illumination. (c) The lamellar system occupies most of the stroma. The size and number of osmophilic granules has increased. X 32,500.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Greening of 10-day-old etiolated barley seedlings.

The sequence of structural changes observed in the plastids of 10-day-old etiolated seedlings of normal and mutant barley subjected to light of different duration are shown in Fig. 11 to 17. The morphological steps involved in the transformation of etioplasts to chloroplasts in 10-day-old normal barley during greening were similar to those observed in the 7-day-old etioplasts. On illumination, the membrane material of the prolamellar body dispersed in the stroma and formed primary layers. Secondary and tertiary layers appeared on these primary layers to form grana. The complete architecture of the chloroplasts was formed within a period of 12 - 24 hr of illumination, whereas in the 7-day-old seedlings the process of transformation was somwhat slower and chloroplasts were formed within a period of 1 to 2 days of illumination.

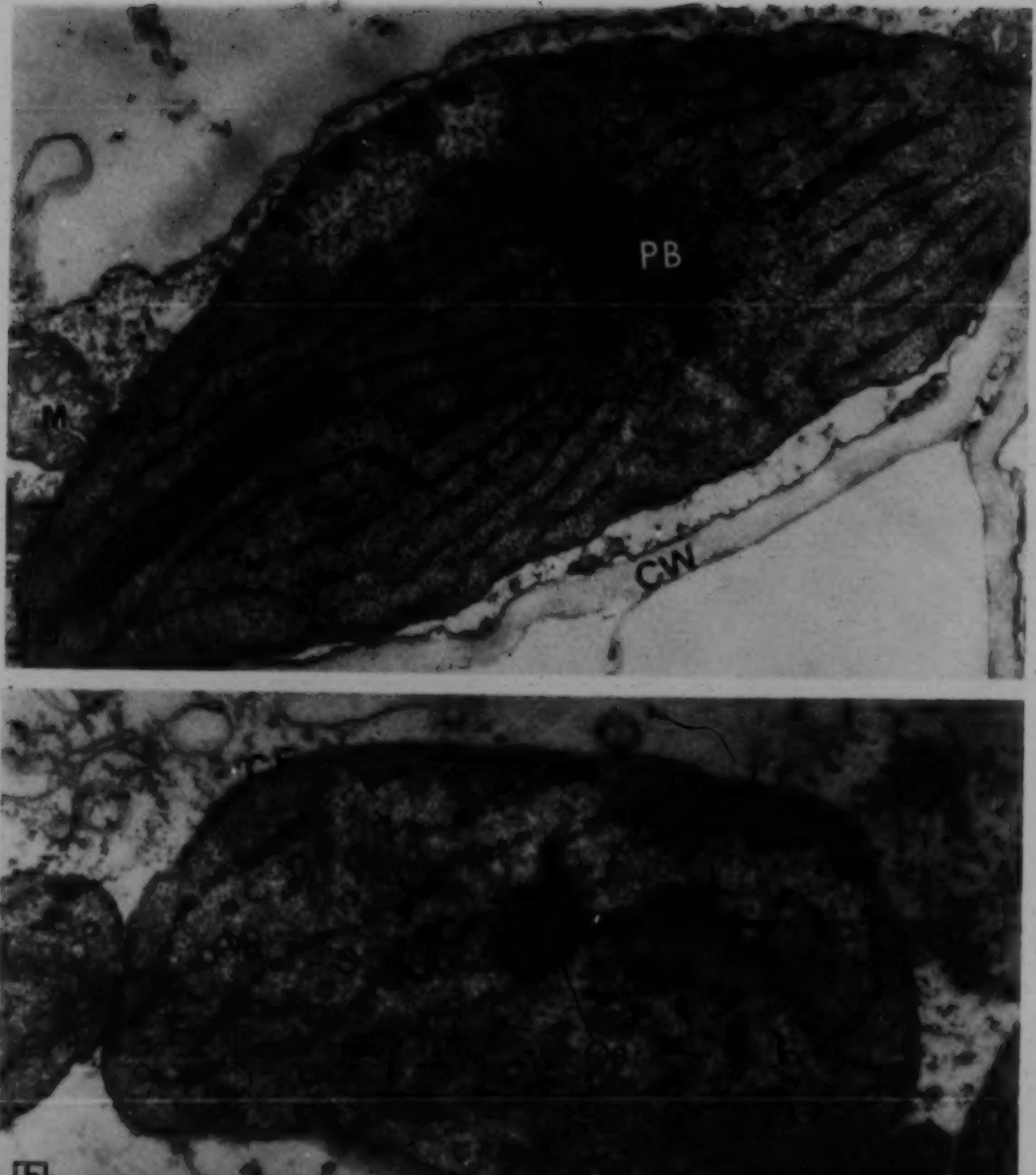
Upon illumination plastids of 10-day etiolated seedlings of mutant barley developed into normal appearing chloroplasts faster than those of the 7-day seedlings. During greening, the plastids of 10-day normal and mutant barley seemed better developed at all the stages than those of the 7-day-old seedlings.

The plastids of the seedlings of normal barley exposed to 1 hr of light followed by a 1 hr dark period contained diffused prolamellar bodies (Fig. 11a). The latter contained large numbers of osmophilic granules. During this period the dispersal of membrane material of the

FIGURE 11. Plastids from barley seedlings grown in the dark for 10 days and then exposed to light for 1 hr followed by a 1 hr dark period.

(a) Normal. A prolamellar body is present in the plastid and contains many osmophilic granules (Os). A number of primary thylakoids radiating out of the crystalline prolamellar body are present. X 34,860.

(b) Mutant. A plastid in which numerous vesicles are present and some are forming thylakoids. Concentrated in the center are osmophilic granules. Some unidentifiable material strongly stained with OsO_4 is shown (arrow). X 43,200.


PB
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b

prolamellar body and the formation of primary layers was in progress. The presence of prolamellar bodies in the plastid after 1 hr of illumination may have been due to the reformation of the prolamellar body in the subsequent dark period. In some plastids no prolamellar bodies were observed.

In comparison to plastids of 7-day-old etiolated seedlings of the mutant illuminated for 1 to 3 hr those of the 10-day-old seedlings were bigger in size, more differentiated and, although the stroma appeared more like that of the normal plastids it also contained many vesicles of varying sizes. Fig. 11b shows a representative plastid at this stage, in which many osmophilic granules are seen concentrated near the center. The arrow indicates some unidentifiable material which stained heavily with osmium.

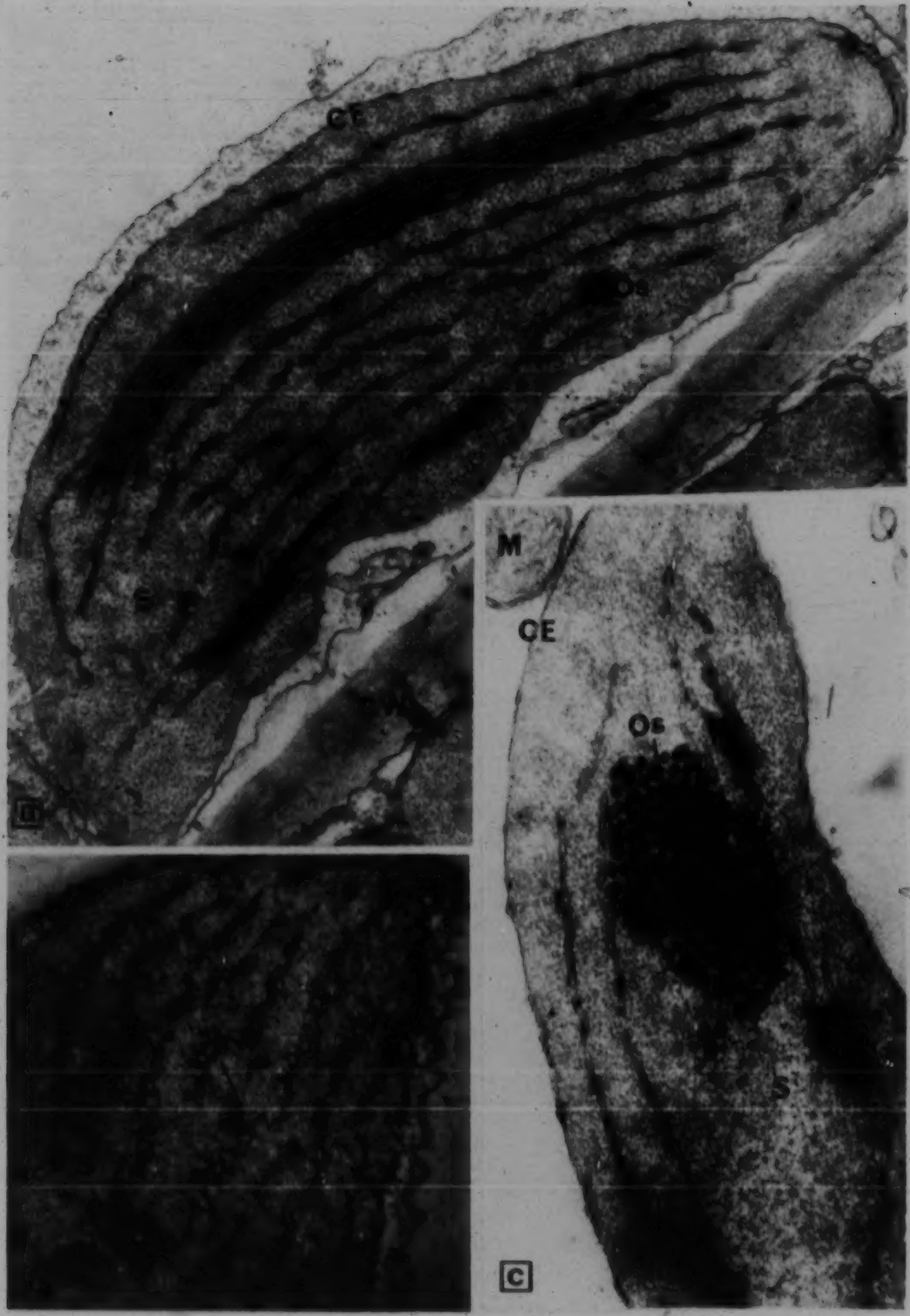
After illumination of the seedlings for 3 hr followed by a 3 hr dark period, normal barley plastids showed primary lamellae which were arranged more or less parallel to each other (Fig. 12a). These primary thylakoids are in the form of double membrane sheets and contain many perforations (Gunning and Jagoe, 1967). Fig. 12b shows perforations of such sheets when sectioned obliquely. The spaces between perforations appear to be equidistant. A few plastids showed a group of osmophilic granules and two or three primary layers (Fig. 12c). The plastids which had many osmophilic granules contained fewer primary layers. This may indicate that these osmophilic granules could

FIGURE 12. Plastids of normal barley grown in the dark for 10 days and then exposed to light for 3 hr followed by a 3 hr dark period.

(a) Plastid with primary thylakoids arranged in parallel arrays. X, 32,500.

(b) Double membrane sheets of a plastid showing perforations. X 23,800.

(c) Plastid with a few primary layers and numerous osmophilic granules. X 32,500.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serve as building material for the primary layers.

Plastids of mutant barley showed numerous vesicles and small thylakoids (Fig. 13a) the latter were observed joining together. Some plastids of the same and adjacent cells appeared much like normal plastids (Fig. 13a, NC). Sometimes an area of the section showed normal chloroplasts while in another the chloroplasts were abnormal. Some small vesicles between which heavily stained grana-like material was noted are shown in Fig. 13b. This material was not observed in the vesicles of 7-day-old dark grown mutant plastids during greening.

On illumination of normal seedlings for 6 hr followed by a 3 hr dark period secondary layers were formed on the primary layers. In some plastids tertiary layers were also formed. A few plastids contained osmophilic granules. The developmental changes observed in the plastids of all the mesophyll cells of the leaf were not synchronized. Fig. 14a shows a plastid of this stage which has formed secondary layers. A group of osmophilic granules is also present in this plastid. Mt indicates microtublue-like structures of chloroplasts which were sectioned longitudinally. A cross section of these structures is shown in Fig. 14b. These structures are discussed in detail beginning on page 106 of the thesis. Reformation of crystalline prolamellar bodies have been reported in the plastids of *Avena* leaf illuminated for 5 hr followed by a 30 min dark period (Gunning and Jagoe, 1967). Such plastids showed grana as

FIGURE 13. Plastids of mutant barley grown in the dark for 10 days and then exposed to light for 3 hr followed by a 3 hr dark period.

(a) Plastids containing vesicles (V) thylakoids (T) and osmophilic granules (Os). Some heavily stained material sandwiched between vesicles (arrows). A normal type of plastid containing primary layers is seen in the adjacent cell (NC). X 37,000.

(b) Part of a plastid in high magnification showing a transformed prolamellar body (PB), osmophilic granules (Os) and grana formation from vesicles (arrow). X 79,200.

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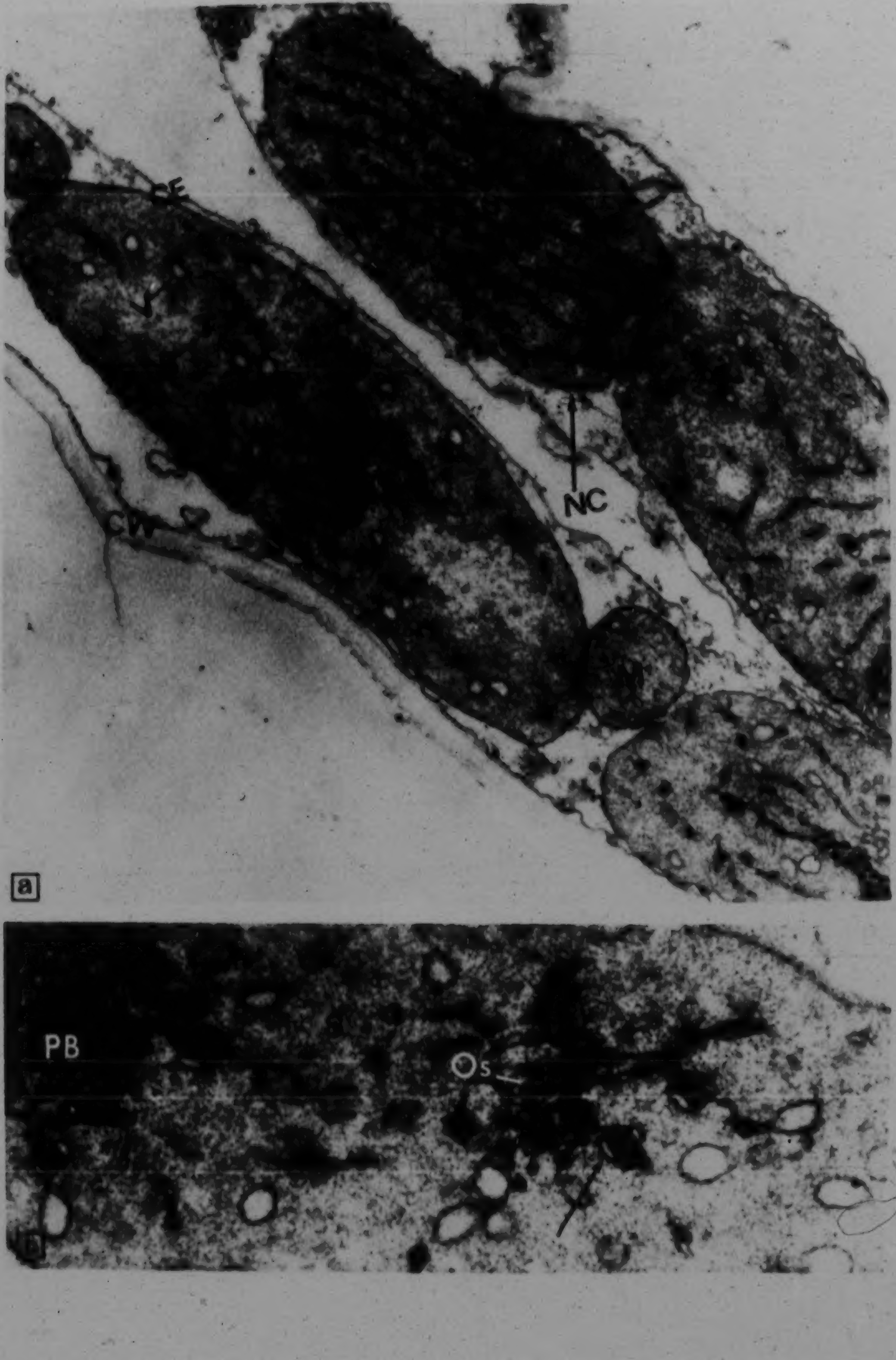
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well as crystalline prolamellar bodies. In the study of Gateway barley no reformed crystalline prolamellar bodies were observed in the plastids of seedlings illuminated for 3 hr and 6 hr followed by a 3 hr dark period. However, a few plastids of the mutant showed prolamellar bodies when illuminated for 3 hr followed by 3 hr of darkness.

Plastids of mutant barley seedlings exposed to light for 6 hr followed by 3 hr of darkness did not show significant changes from those illuminated for 3 hr followed by a 3 hr dark period. They still contained vesicles. Fig. 14c shows a plastid of this stage which consists of a few primary layers. Some vesicles are seen at the ends of these layers.

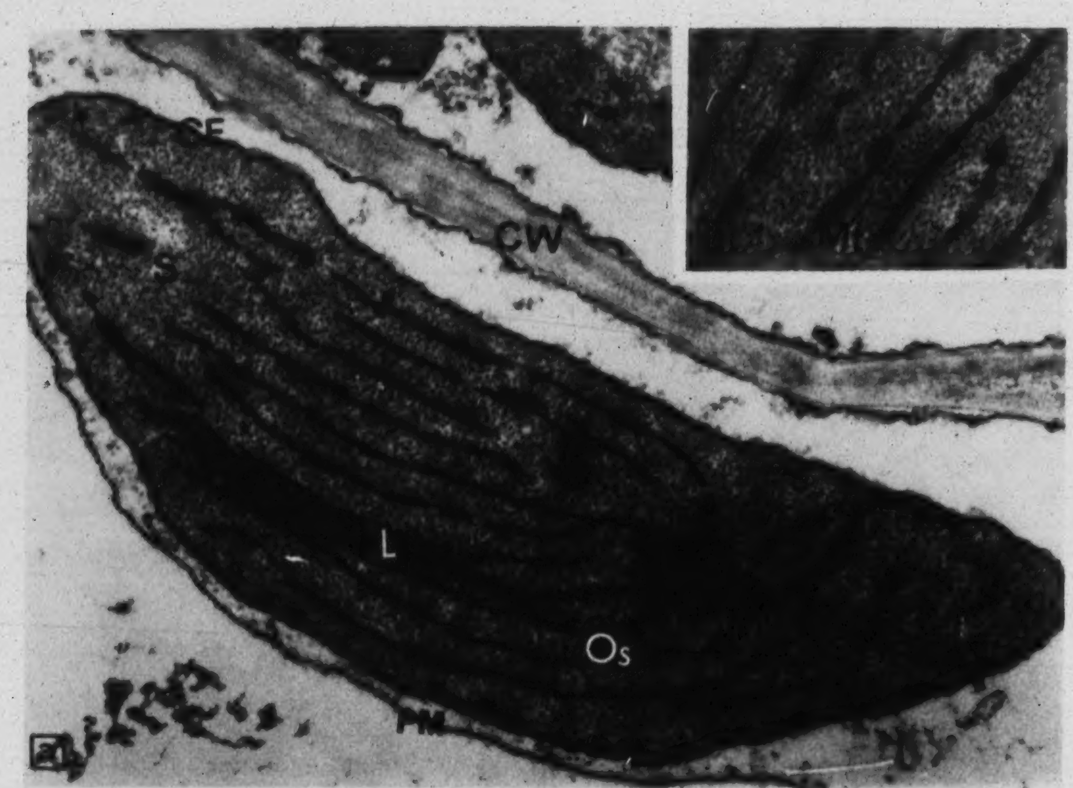
On illumination of the normal seedlings for 12 hr followed by 3 hr darkness, grana were formed in some plastids and in 24 hr illuminated seedlings all the plastids were well developed. Typical plastids of this stage are shown in Fig. 15a and b. Some chloroplasts contained starch grains (Fig. 15b). A high magnification of grana lamellae is seen in Fig. 15c which shows loculi and partitions of the grana (Weier and Benson, 1966).

Grana formation occurred in the plastids of mutant barley after illumination for 2 days. Fig. 16a shows a plastid at this stage. Some vesiculated thylakoids are extending out of the grana into the stroma. Presumably these would form stroma lamellae. A large starch grain surrounded by grana stacks is seen. Within a period of 4

FIGURE 14. Plastid structure of 10-day-old dark grown Gateway and the mutant exposed to light for 6 hr followed by a 3 hr dark period.

(a) and (b) Normal. Secondary layers are seen on the primary layers. Os - osmophilic granules, Mt - microtubule-like structure, (a) sectioned longitudinally and (b) in cross-section. X 32,400.

(c) Mutant showing formation of primary layers. Typical vesicles are seen as well as some with lamellar-like stacks. X 42,300.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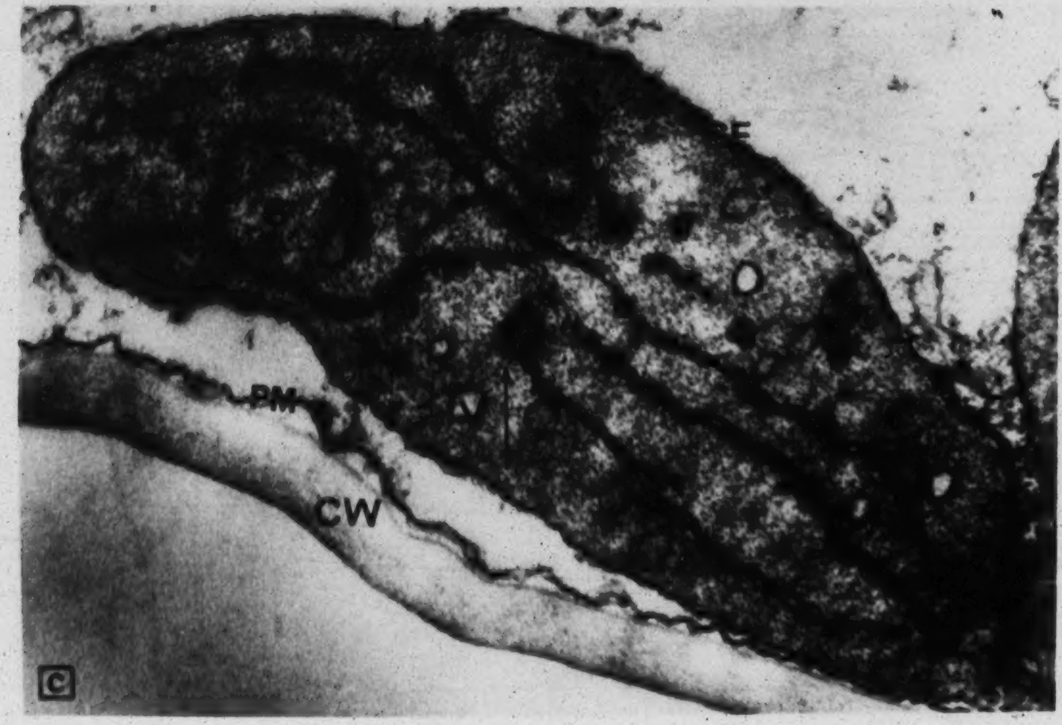
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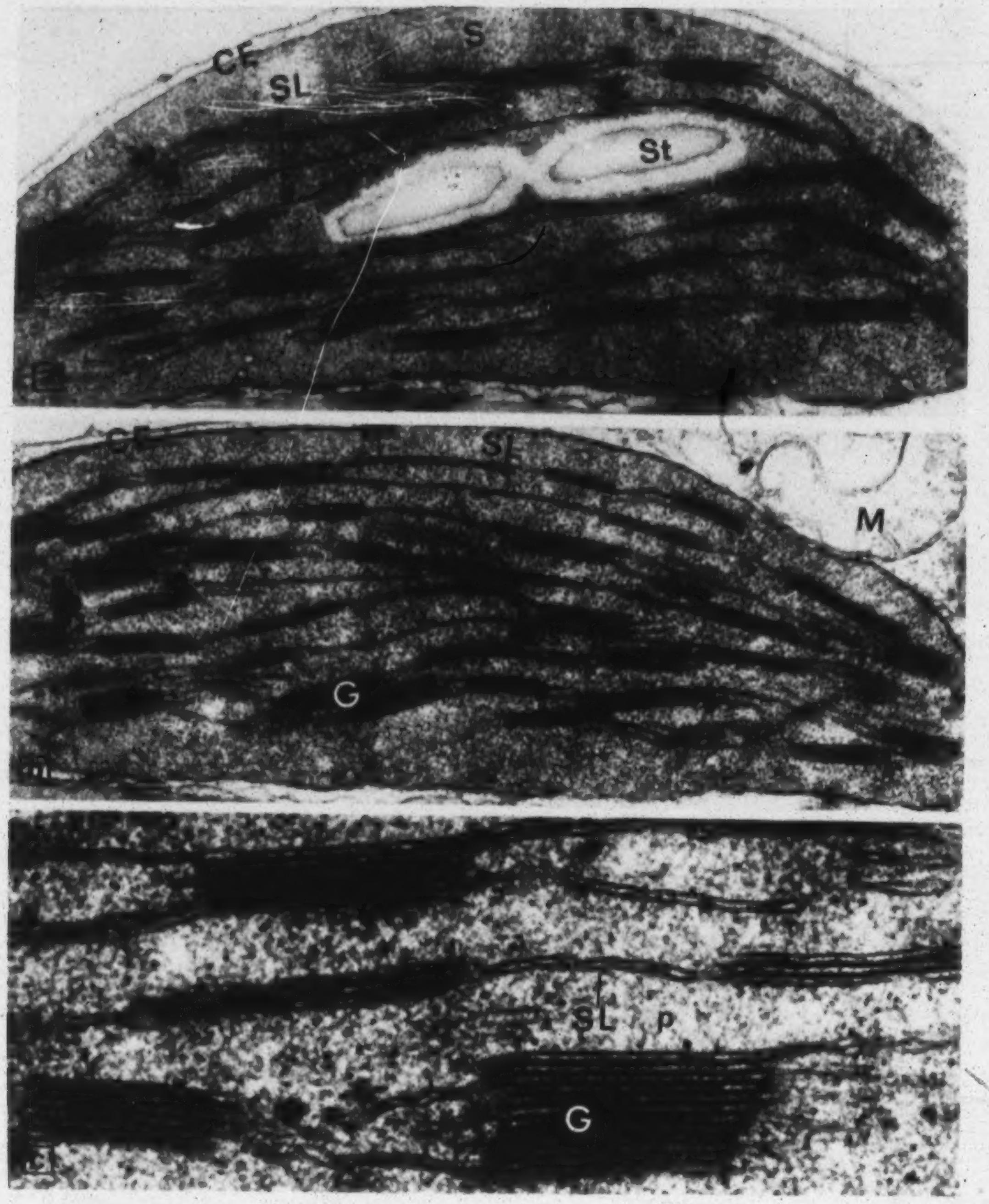
FIGURE 15. Chloroplasts of normal barley grown in the dark for 10 days and then exposed to light for 24 hr followed by 12 hr dark.

Well developed chloroplast showing grana (G) stroma (S) osmophilic granules (Os).

(a) with starch grains (St). (b) without starch grains. X 35,500.

(c) Grana showing loculi and partitions under high magnification. X 97,000.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to 6 days of illumination chloroplasts of the mutant contained characteristic features similar to those of normal barley in that they contained grana as well as stroma lamellae.

The formation of stroma lamellae was in progress in 4-day illuminated seedlings (Fig. 16b) and by 6 days of illumination many stroma lamellae were formed (Fig. 17). Bundles of microtubule-like structures were seen in some plastids (Fig. 16c). A high magnification electron micrograph of these tubules sectioned longitudinally is shown in Fig. 16d.

Seedlings grown under continuous illumination of 1400 ft-c.

Chloroplasts of normal seedlings grown under these conditions for 4 days were well developed and showed a structure that is typical for chloroplasts from higher plants (von Wettstein, 1958). They were bounded by a double membrane envelope and contained a lamellar system which was embedded in the granular stroma (Fig. 18a). The grana were made up of 2-12 thylakoid discs. Some of the discs of the grana continued to extend in the stroma and connected with adjacent grana. These are referred to as stroma lamellae. Osmophilic granules were also seen in the stroma. In this experiment the plants were left in darkness overnight which may account for the chloroplasts being depleted of starch. All the plastids in the sections examined were uniformly well developed.

FIGURE 16. Plastids from mutant barley seedlings grown in the dark for 10 days and then exposed to light for various periods of time.

(a) Illuminated for 2 days followed by 12 hr dark. Plastid with a number of grana lamellae surrounding a starch grain (St). The stroma lamellae extending out of the grana are vesiculated. X 47,700.

(b) and (c) 4 days illumination followed by 12 hr dark.

(b) Plastid contains grana some of which are connected with one another by stroma lamellae. X 34,500.

(c) Long bundle of microtubule-like structures in the plastid. X 56,000.

(d) High magnification of a portion of c. X 73,500.

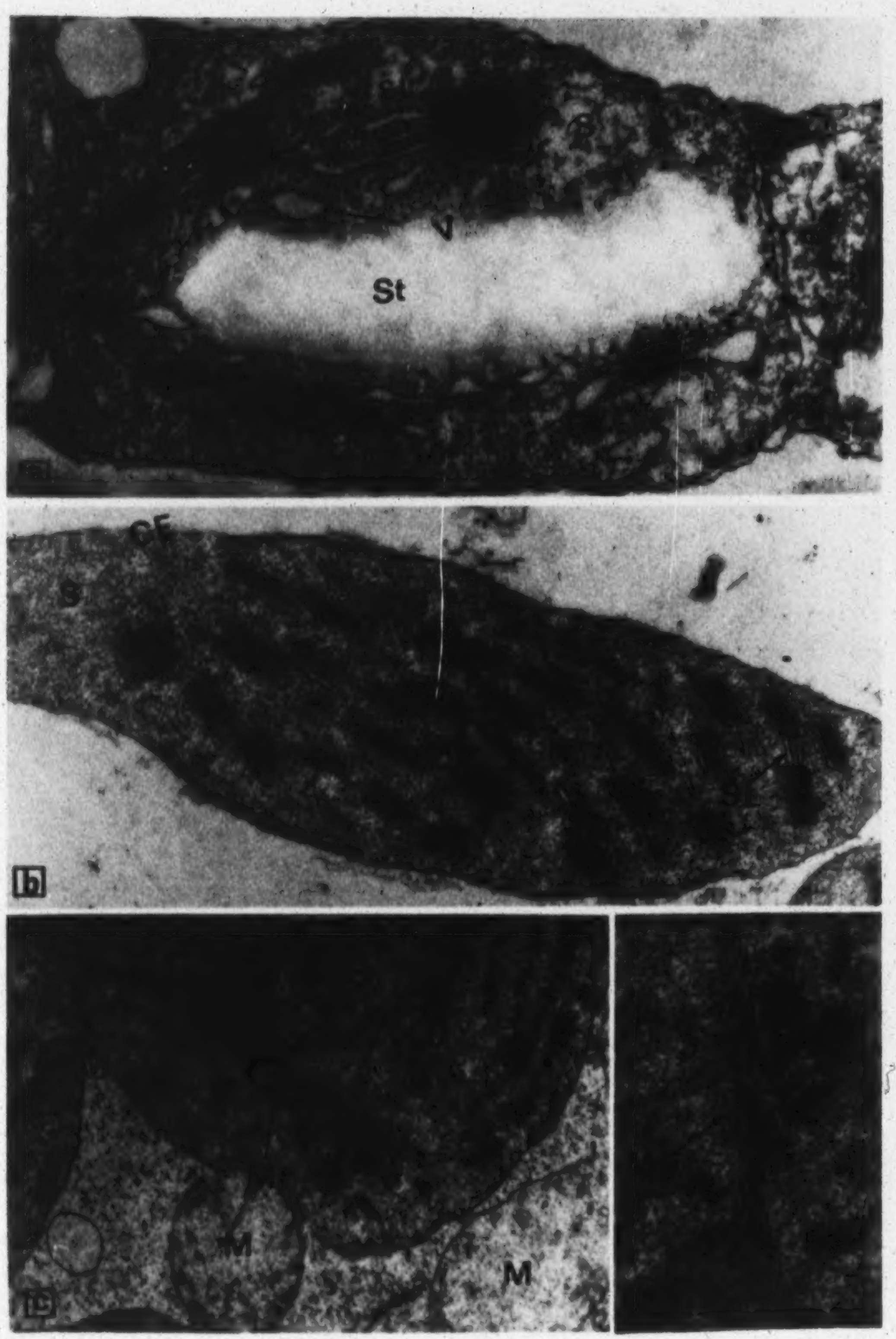
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FIGURE 17. Plastid of 10-day-old dark grown mutant seedling illuminated for 6 days.

A well developed plastid with grana and stroma lamellae. X 32,400.

Normal barley plastids from 6- and 8-day-old seedlings were not very different in structure from those of the 4-day ones except that they were slightly bigger and the granal discs were more numerous (Fig. 18b).

There was a great deal of variability in the structure of plastids from 4-day-old seedlings of the mutant barley. None of the plastids in the sections examined were as well developed as the plastids of 4-day-old normal barley. The majority of plastids were smaller in size and there was no organization of the lamellar system. They lacked thylakoids and contained big vesicles (Fig. 19a). Other plastids were at different juvenile stages of development. Fig. 19c shows a plastid with an aggregated mass of tubules of the prolamellar body. Many osmophilic granules are interspersed in the prolamellar body. An area of low electron density containing DNA fibrils can be seen in the plastid stroma (arrow). Such areas consisting of DNA have also been reported in *Chlamydomonas* (Ris and Plaut, 1962), *Avena* (Gunning, 1965b) and *Beta vulgaris* (Kislev *et al.*, 1965).

Dispersal of the tubules of the prolamellar body in the stroma was also observed (Fig. 19d). Membrane material of the prolamellar body was dispersed leaving osmophilic granules at the place of the prolamellar body (Fig. 19b to e).

Tube transformation and tube dispersal stages observed in some plastids of mutant barley suggest that

FIGURE 18. A typical chloroplast of 4-day (a) and 8-day-old normal barley (b) grown in continuous light of 1400 ft-c, showing grana (g) and stroma lamellae (SL).

(a) X 32,700. (b) X 34,000.

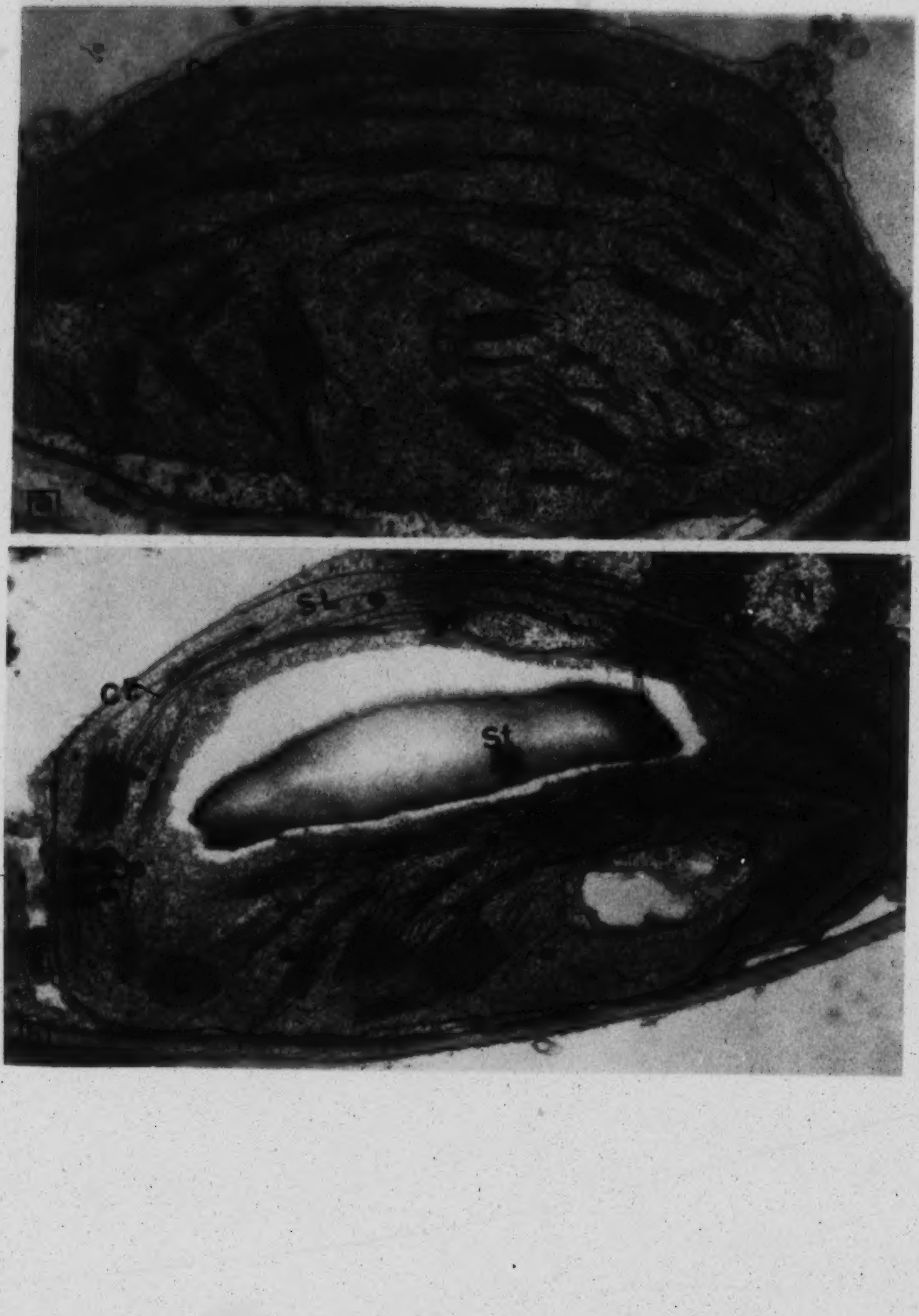
a
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FIGURE 19. Sections of 4-day-old mutant barley seedlings grown under continuous light showing a range of plastid variability.

(a) Plastids containing big vesicles (V). X 40,700.

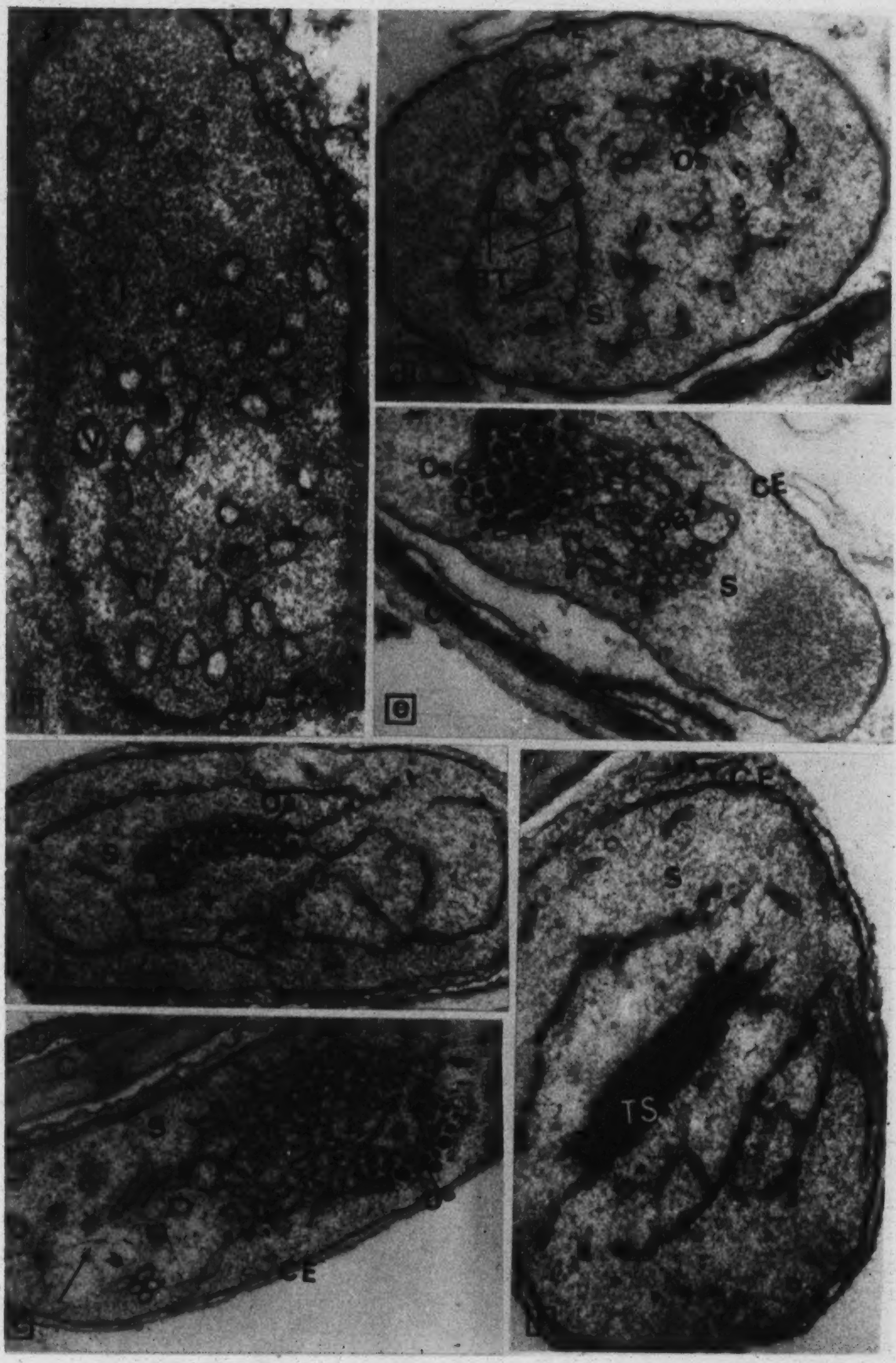
(b) Plastid with a few thylakoids and many osmophilic granules (Os). X 24,600.

(c) Part of a plastid showing an aggregated mass of tubules presumably formed from the crystalline prolamellar body. X 33,800.

(d) Membrane tubules of the prolamellar body are dispersed in the plastid. X 36,700.

(e) Plastid showing tubules of prolamellar body (PBT) and osmophilic granules. X 43,300.

(f) Plastid showing stacked lamellae (pseudo-grana structure). X 36,200.



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plastids formed prolamellar bodies even in continuous light. Occasionally a mass of abberant tightly packed lamellae was observed in the plastid giving the appearance of pseudo-grana structures (Bachmann *et al.*, 1969).

Fig. 20 shows plastids of 6-day-old seedlings of mutant barley. These plastids had not developed primary thylakoids or grana but contained vesicles of variable sizes. From the observation of many plastids one can infer the changes which may have occurred in the vesicles to form grana. The vesicles which were surrounded by a membrane were either electron transparent (Fig. 20a) or granular (Fig. 20d). A few vesicles in the plastids showed a peculiar electron dense massive structure which had stained heavily with osmium (Fig. 20b). Some plastids also contained aggregated tubules which may have represented transformed prolamellar bodies like in the plastids of 4-day-old mutant seedlings. The vesicles observed did not seem to be formed by swelling of the grana as has been described in a thiamine requiring tomato mutant (Boynton, 1966). These vesicles aggregated (Fig. 20, e,f,g) flattened to form discs and joined together side by side to form grana. Sometimes they joined end to end to form primary lamellae. Fig. 20f shows some vesicles adjoining a granum. All the plastids at the 6-day stage contained vesicles.

Fig. 21 shows typical chloroplasts of 8-day-old mutant seedlings. The chloroplasts were better developed at this age and very few plastids showed vesicles.

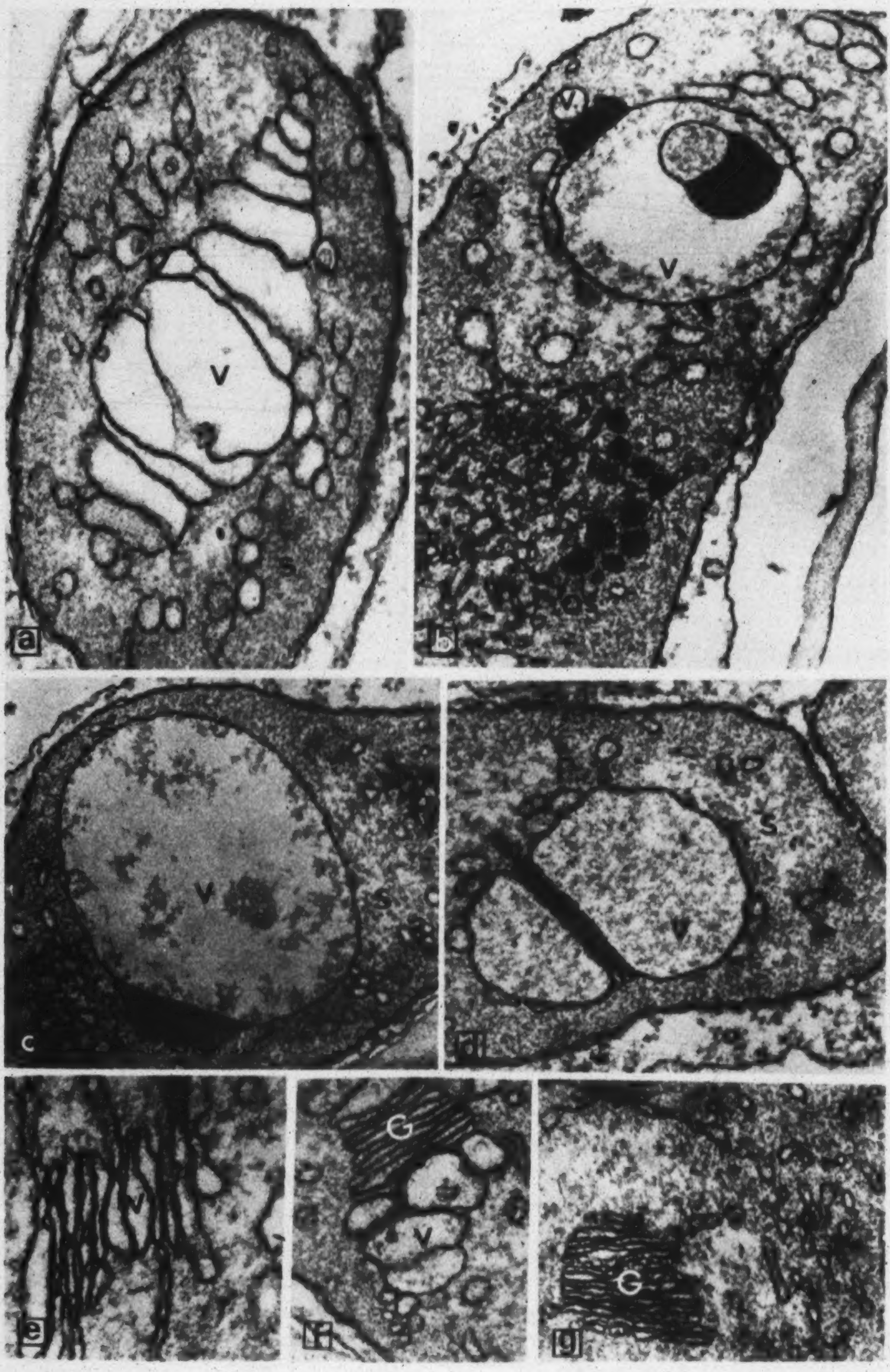
FIGURE 20. Plastid from the seedlings of 6-day-old mutant barley at different stages of development.

(a) Plastids containing vesicles of variable sizes. X 50,400.

(b) Part of a plastid showing vesicles and an aggregated mass of tubules. Some heavily stained electron-dense material is seen in the large vesicle and a few lamellae are attached on its outside. X 48,000.

(c) and (d) Part of a plastid showing large vesicles. Lamellar material is seen outside or in between the two vesicles. (c) X 54,800. (d) X 56,000.

(e), (f) and (g) Process of formation of grana from the vesicles. (e) X 51,000. (f) X 34,000. (g) X 49,300.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Most of the vesicles which were prominent structures in chloroplasts of 6-day-old seedlings had disappeared and the amount of lamellar material had increased. The structure and distribution of grana and their arrangement relative to the plastid surface was not like that of normal chloroplasts. Fig. 21b shows a plastid containing numerous small thylakoid discs some of which were joined together to form grana. The thylakoid discs of the grana were smaller in size and were relatively fewer in number than in the normal. Some grana had normal morphology and contained normal disc compartments which were not seen as clearly as in the normal (see Fig. 15c and 18). Each disc compartment had a loculus and the discs were joined to form partitions. Some grana were made up of tightly packed discs which did not show loculi (Fig. 21). More often concentrated globular masses of material approximating the size of grana were dispersed throughout the stroma. A few lamellae originating from these masses extended into the stroma and some of them were seen joining with other grana.

In normal chloroplasts most grana alternate with stroma lamellae and are connected with them to form rows. There are a number of such rows which have more or less parallel arrangement with respect to the plastid surface. Unlike this the arrangement and distribution of grana in the mutant was irregular. Therefore, in the mutant the interconnecting stroma lamellae ran irregularly in many directions. They were smaller in length and only some of

FIGURE 21. Chloroplast of 8-day-old mutant barley at different stages of development.

(a) Plastid with a few vesicles and small grana. X 27,000.

(b) Part of a plastid with many small thylakoids, some grana consisting of only two or three small discs, and two stacks of tightly packed thylakoid discs. X 32,400.

(c) A plastid showing grana arrangement. Many stroma lamellae (SL) are extending out of the grana. X 27,000.

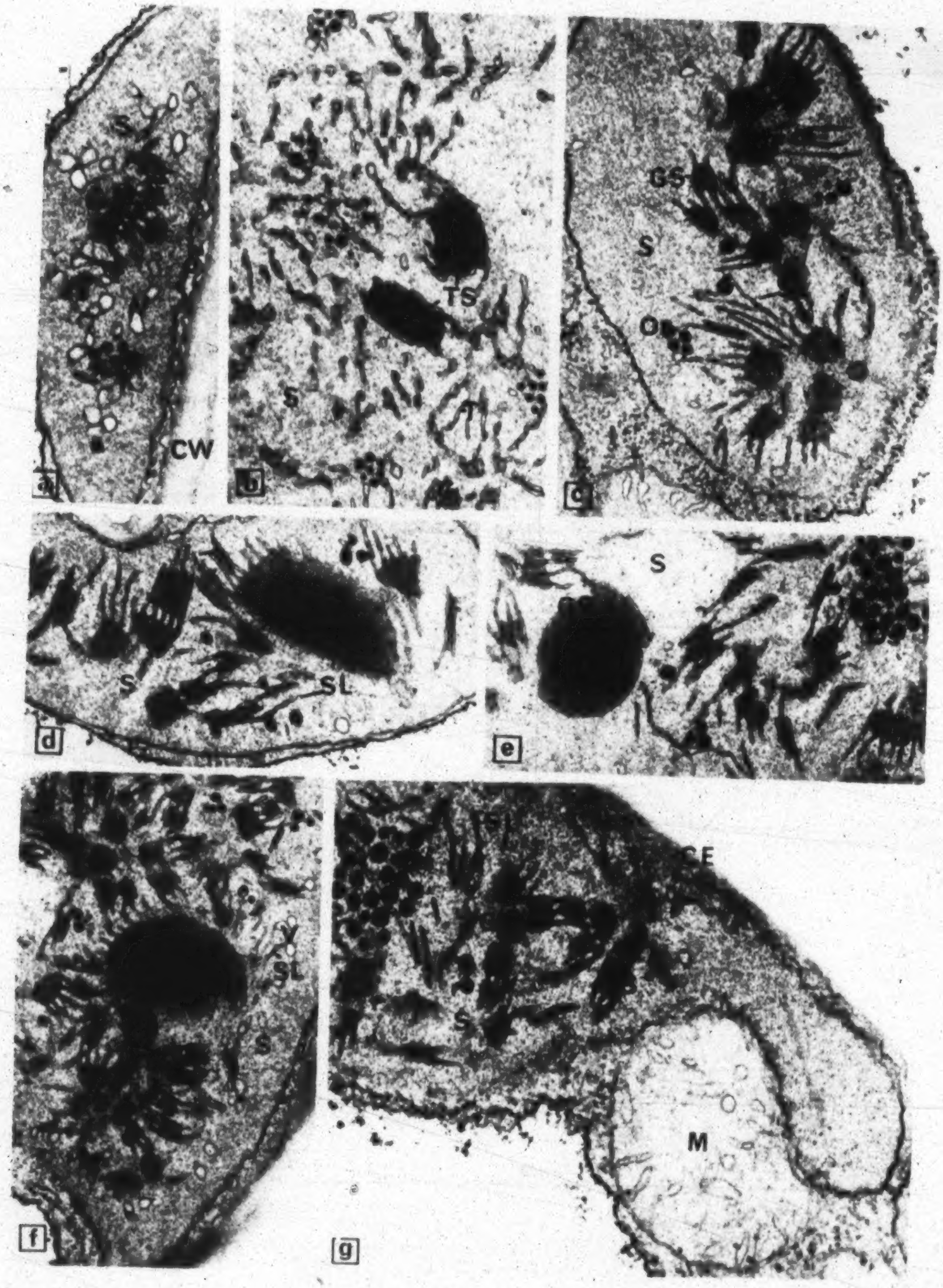
(d - f) Parts of plastids showing abnormal arrangement of lamellar material.

(d) concentric. X 21,600.

(e) longitudinal. X 32,400.

(f) spherical. X 24,700.

(g) Tapered tail plastid. X 24,800.



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them joined pairs of grana. In some instances a large amount of the lamellar material was aggregated into a spherical (Fig. 21f) concentric (Fig. 21e) or longitudinal forms (Fig. 21d) giving a massive appearance. Chloroplasts of the mutant contained some normal grana, as well as abnormally arranged lamellar material. Many osmophilic globules were seen concentrated either in the center or distributed throughout the stroma.

Seedlings grown under continuous illumination of 600 ft-c.

When grown under the lower light intensity of 600 ft-c at 20±1°C the development of chloroplasts of normal barley was similar to that observed in the plants grown under the higher light intensity. The chloroplasts had a well developed lamellar system consisting of grana and stroma lamellae at all ages. However, under these growth conditions the development of the chloroplasts in the mutant barley was different from those in seedlings grown under the higher light intensity. Although the development was slower than in normal chloroplasts it passed through similar stages to those usually observed in normal plants during the development of proplastids into chloroplasts under continuous illumination (von Wettstein, 1958). At no stage did these chloroplasts show the abnormalities which were observed in the plastids grown under the higher light intensity. For example, the plastids of 4-day-old mutant seedlings grown under 600 ft-c did not have vesicles which were a common feature of seedlings grown at 1400 ft-c.

FIGURE 22. Developing plastids from seedlings of mutant barley grown under 600 ft-c at 20°C.

(a) A typical plastid from 4-day-old seedlings containing a few thylakoids. Doubling of a part of a thylakoid which might have arisen by invagination process is seen below the grana (G). X 52,700.

(b) Chloroplast from 6-day-old seedlings containing a few grana and stroma lamellae. A DNA-like region containing DNA fibrils is visible. X 32,500.

(c) Well developed chloroplast from 8-day-old seedlings with grana and stroma lamellae. X 42,500.

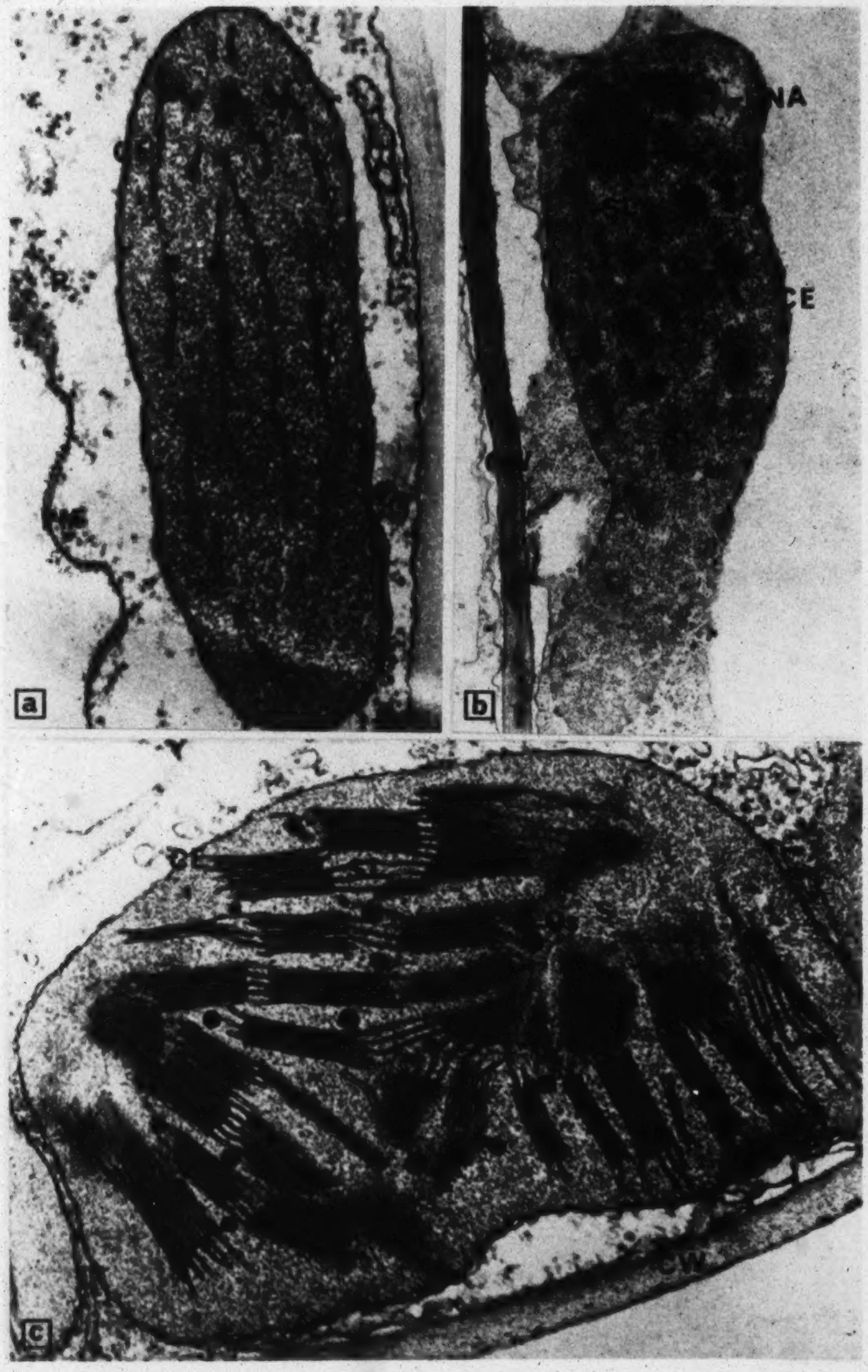
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Fig. 22a shows a typical plastid from 4-day-old mutant seedlings containing a few lamellae in which the process of multiplication is in progress. In this figure the lamellae below the granum show multiplication which could have arisen by an invagination process (Menke, 1962). By 6-days plastids formed small grana which, although fewer in number, were arranged like those of normal chloroplasts. The stroma lamellae were just beginning to form in these plastids (Fig. 22b). Plastids of mutant seedlings at 8-days contain well developed grana and stroma lamellae similar to normal plastids (Fig. 22c). The plastid in Fig. 22b has a DNA-like region in which the fibrils correspond to DNA strands described by other workers (Kislev *et al.*, 1965; Yokomura, 1967; Herrmann and Kowallik, 1970).

Discussion

Maclachlan and Zalik (1963) showed that the virescent mutant of barley was deficient in chloroplast pigments early in development but attained nearly normal levels as growth proceeded. These findings were confirmed in this study and it was seen that under different growth conditions the etioplasts and chloroplasts of the mutant seedlings were smaller in size and less developed than in the normal. During early development plastids were deformed and showed a number of abnormalities. In studies during greening as well as in continuous light a time lag was observed in the formation of the lamellar system in the mutant when compared to the normal. Mego and Jagendorf (1961) found that in black valentine bean during greening there was an increase in the amount of pigments, proteins and lipids in the plastids. Therefore, the time lag observed in the mutant was probably due to the slow synthesis of some chloroplast component. It was found by Maclachlan and Zalik (1963) that accumulation of protochlorophyll and chlorophyll in the mutant was slower than in the normal. Sane and Zalik (1970) from studies on metabolism of ^{14}C-acetate and ^{14}C-leucine concluded that the mutant was 5 times less efficient at 6 days but only 1.5 times less efficient than the normal at 10 days.

The mutant contained a variety of plastids ranging from those with a few lamellae or prolamellar bodies to normally developing plastids. In many of its characteristics the mutant resembled a one gene virescent mutant of maize (Chollet and Paolillo, 1972) which was deficient in pigments and contained a variety of different plastids in ungreened leaves but well developed chloroplasts in green leaves. Studies on other viable mutants have indicated that although they contained well developed chloroplasts they had a relatively low amount of lamellar aggregation (Bennedict and Kohal, 1970; Clewell and Schmid, 1969; Dale and Heyes, 1970; Highkin *et al.*, 1969).

Etiolated seedlings of the mutant contained only 65 to 85% of normal amounts of protochlorophyll (Maclachlan and Zalik, 1963) and electron microscope studies have shown that most of the etioplasts were undifferentiated and contained vesicles or inconspicuous prolamellar bodies. It has been reported previously that the accumulation of protochlorophyll(ide) coincides with the formation of prolamellar bodies (von Wettstein and Khan, 1960; Gunning and Jagoe, 1967; Henningsen and Boynton, 1969) and is required for their formation (Henningsen and Boynton, 1970; Khan, 1968a). A mutant of barley, *xantha-10*, which had a block between protoporphyrin IX and protochlorophyll failed to form crystalline prolamellar bodies (von Wettstein, 1959) and a temperature sensitive mutant of *Zea mays* containing low protochlorophyll also failed to organize the elements of the prolamellar body tubules into a crystalline array (Millerd *et al.*, 1969). However, etioplasts of carotenoid-deficient mutants of maize contained normal crystalline prolamellar bodies (Bachmann *et al.*, 1967; Troxler *et al.*, 1969). These studies suggested that protochlorophyll(ide) is an indispensible component of the crystalline prolamellar bodies. Failure to form the crystalline prolamellar bodies in the presence of 65 to 80% of the normal amount of protochlorophyll in the present virescent mutant suggests that it suffers from a deficiency of components other than protochlorophyll. Boardman and Anderson (1962) found that protochlorophyll(ide) was bound to the holochrome protein and Khan (1968a) and Lafleche *et al.* (1972) have suggested that holochrome protein is a structural component of the tubular membranes. Thus it appears that the formation of crystalline prolamellar bodies did not depend solely on protochlorophyll. It is possible that crystalline prolamellar bodies in the mutant were not formed because of the lack of protochlorophyll-holochrome protein. From the results of incorporation studies (Sane and Zalik, 1970) it was suggested that the mutation had caused a partial inhibition of synthesis of holochrome protein.

Since most plastids of the mutant lacked prolamellar bodies, the normal sequence of changes which were observed during transformation of prolamellar bodies to form lamellar systems in the chloroplasts of the normal must not have occurred in the mutant. Thus the development of the mutant took a different course than the normal. The number of vesicles in the mutant increased upon illumination and eventually these were incorporated along with newly synthesized material to form grana and stroma lamellae. The development of grana in the two lines was different. In this respect the mutant differs from the virescent mutant of *Phaseolus vulgaris* in which prolamellar bodies were transformed in the same way as its wild type (Dale and Heyes, 1970).

The crystalline prolamellar bodies of the normal barley plastids showed a similar progressive sequence of structural changes upon illumination as has been reported previously for normal barley (von Wettstein, 1958; Henningsen and Boynton, 1970). Small vesicles which formed during tube transformation gave rise to perforated lamellar sheets, however, vesicles did not disperse into stroma as has been observed in beans (von Wettstein and Khan, 1960). Vesicle formation during tube transformation was not observed in tobacco and oat seedlings (Boasson *et al.*, 1972; Gunning, 1965a). The differences observed could be due either to the difference in species or to the conditions under which they were grown. In bean leaves Sironval *et al.* (1969) noted formation of big vesicles from prolamellar bodies when illuminated by intermittent flashes. It appears that in normal plants biosynthesis and development of structure is faster in some species than in others and may be affected by the growth conditions.

Thus if the rate of development is rapid discrete steps in biogenesis of membranes might not be discerned.

Dark grown seedlings of the mutant or seedlings grown at 600 ft-c lacked prolamellar bodies, however, they were formed in plants grown in continuous light at 1400 ft-c. It seems that these prolamellar bodies passed through a similar but slower progressive sequence of changes as were observed in the prolamellar bodies of normal barley during greening.

Grana formation in the normal seemed to occur through the multiplication of primary layers by an invagination and protrusion process (Menke, 1962; Wehrmeyer and Röbbelen, 1965). According to Wehrmeyer and Röbbelen (1965) the protusion process can give rise to six types of appearance of lamellae in sections of plastids depending upon where they are cut. The lamellae in Figure 6 show some of these types of appearances. The present detailed investigation of development in the mutant showed that the primary layers were absent and the vesicles appeared to flatten and join together forming grana. It is possible, however, that grana were formed by a process of invagination from the vesicles, a mechanism similar to their formation from the primary layers in normal plastids. The presence of compartmented vesicles in the mutant was taken as evidence for this, and would mean that the vesicles were not formed by the swelling of grana as was thought previously (Maclachlan and Zalik, 1963). Grana formation in the mutant occurred before stroma lamellae were formed. Formation of grana lamellae before stroma lamellae has also been recently reported in a virescent mutant of maize (Chollet and Paolillo, 1972).

Miller (1965) reported that total RNA content of the mutant leaves was about 75% of that present in the normal. It is very probable that the lower amount of RNA was due to a deficiency of chloroplast ribosomes. In electron micrographs ribosomes were not well resolved, however, it was seen that etioplasts of the mutant lacked crystalline prolamellar bodies which are formed in the presence of ribosomes (Gunning and Jagoe, 1967). Also the stroma of the juvenile chloroplasts was occupied by large vesicles. Involvement of chloroplast ribosomes in the synthesis of membrane proteins has been suggested by a number of workers (Shumway and Weier, 1967; Falk, 1969). By studying a mutant of *Chlamydomonas reinhardi*, Goodenough and Levine (1970) found that proper membrane organization, PSII activity and RuDP carboxylase activity were dependent on the presence of chloroplast ribosomes. Thus it is possible that slower membrane formation in the mutant is due to a deficiency of chloroplast ribosomes. Srivastava *et al.* (1971) studied the effect of chloramphenicol on membrane transformation in *Pisum sativum* chloroplasts. Their results suggested that grana formation may be regulated by protein(s) synthesized on the plastid ribosomes, whereas the formation and maintenance of prolamellar bodies as well as synthesis of plastid membranes may be regulated by proteins synthesized in the cytoplasm.

Biogenesis of chloroplast membranes has been investigated by Eytan and Ohad (1970) and by Hoober (1970) using a mutant of *Chlamydomonas reinhardi y-1* and antibiotic inhibitors. Their results suggested that there was cooperation between cytoplasmic and chloroplast ribosomes in the synthesis of photosynthetic lamellar proteins during the greening process.

Generally, structural changes observed in chloroplasts under the electron microscope have been correlated with changes in pigments. No specific information is yet available about the lamellar membrane proteins synthesized during greening in higher plants.

One of the objectives of this study was to relate plastid development to changes in chloroplast proteins. Investigation of the chloroplast proteins (given later in this thesis) showed that the mutant lacked certain proteins in early stages of development. However, the large variations observed in the development of plastids within a single cell made it difficult to relate chloroplast structure to the proteins present, since these were averaged over the whole leaf. On the other hand, the presence of aberrant and normal plastids in a single cell, suggested that individual chloroplasts have some degree of autonomy.

Bachmann *et al.* (1969) categorized grana into three types on the basis of chlorophyll content of the leaf. The abnormally large grana in the mutant plastids in Figure 9b correspond to what they designated as non-chlorophyll containing grana. A morphological comparison of the plastids of mutant seedlings at various stages during greening as well as in continuous light of 1400 ft-c indicated that a majority of the chloroplasts were abnormal, some were undeveloped and a few were normal.

The heavily staining material seen in some developmental stages of the mutant appeared to be lipid in nature and might represent an accumulation of some metabolic product which was being utilized slowly.

A comparison of development of chloroplasts in plants grown in darkness for 7 days and for 10 days showed that the 7-day-old seedlings required a longer period of illumination than the 10-day ones. The etioplasts of 10-day-old seedlings were better developed than those of 7 days in both varieties. These results are not in agreement with Henningsen and Boynton (1970) who reported that barley etioplasts reached their maximum size and protochlorophyll(ide) content at 7 days and after that their protochlorophyll(ide) content decreased.

In the present study no specific attempt was made to determine whether slow differentiation of etioplasts into chloroplasts in the mutant involved photodestruction of chlorophyll. Photodestruction of chlorophyll in strong light has been reported in a number of carotenoid-deficient mutants and it has been suggested that carotenoids are required to protect chlorophyll from photodestruction (Anderson and Robertson, 1960; Walles, 1966; Bachmann *et al.*, 1967). As found in earlier studies by Maclachlan (1962) and confirmed by the present work the mutant was deficient in carotenoids at each stage examined. The amount of chlorophyll seemed to be directly related to the amount of carotenoids present. Comparison of chloroplast formation in continuous light at 600 ft-c and 1400 ft-c revealed that plastids from seedlings grown at the higher light intensity were less developed and contained less carotenoids than those grown at the lower light intensity.

Miller (1965) reported that pigment formation in the mutant was sensitive to light intensity and temperature. He found that accumulation of carotenoids increased in the mutant with increase in light intensity and the maximum accumulation occurred at 2600 ft-c and 24.5°C. Conversely, normal barley accumulated more carotenoids at lower light intensity and the maximum amount was formed at 1020 ft-c and 24.5°C. The latter conditions were also optimal for chlorophyll formation in both lines. The apparent difference in requirements for high carotenoid formation in the earlier study (Miller, 1965) may have been due to differences in the growth conditions. Photo periods of 16 hr light and 8 hr darkenss were used and the light source was entirely incandescent lamps, whereas the present study employed continuous cool white fluorescent light.

A number of mutants of *Helianthus* and *Zea* blocked in carotenoid synthesis are known to be sensitive to normal levels of illumination (Walles, 1965, 1966, 1971; Bachmann *et al.*, 1967; Troxler *et al.*, 1969). EM studies showed that in the dark the mutants of *Helianthus annuus* formed etioplasts and ribosomes similar to those in the wild type. They could synthesize and preserve some chlorophyll and ribosomes in dim light (20 lux). However, photodestruction of ribosomes and chlorophyll occurred when they were illuminated at higher light intensity (500 lux). From these results it was suggested that the role of carotenoids was to protect chlorophyll from photodestruction. Furthermore, Walles (1972) suggested that perhaps plastid ribosomes were sensitive to light of wavelengths normally absorbed by carotenoids - specifically β-carotene. It is quite possible that under the conditions used in this study the plastid ribosomes of the virescent mutant were sensitive to the higher light intensity.

II. Endocytotic-like Process in Chloroplasts of the Mutant Barley

Accompanying the abnormal chloroplast development in the mutant a number of peculiarities were observed. The most striking of which was the presence of mitochondria inside the chloroplasts. A few examples of plastids from the mutant containing mitochondria are seen in Fig. 23. Plastids with mitochondria occurred in the mutant grown in continuous light as well as during greening of dark-grown seedlings. But mitochondria were never observed in chloroplasts of the normal barley. The presence of mitochondria inside mature chloroplasts has been previously reported by Vesk *et al.* (1965).

The structure of the mitochondria in the chloroplasts appears similar to the mitochondria in the cytoplasm (Fig. 24e, 21g). In some plastids the mitochondria are encircled by a double membrane vacuole of the chloroplast and a wide space between the two is visible (Fig. 23a and c, arrow). This double membrane of the vacuole is a feature which distinguishes it from vesicles which also occur frequently in mutant plastids (Fig. 24a). The stroma of the chloroplast is separated from the mitochondria by the double membrane of the vacuole which usually appeared at one corner of the chloroplast. Fig. 23d shows a plastid in which part of the mitochondrion is in close association with the stroma of the chloroplast and in this area the membranes are not distinguishable. Fig. 23e and f are

FIGURE 23. Electron micrographs showing mitochondria inside the chloroplasts of the mutant seedlings at various stages of development.

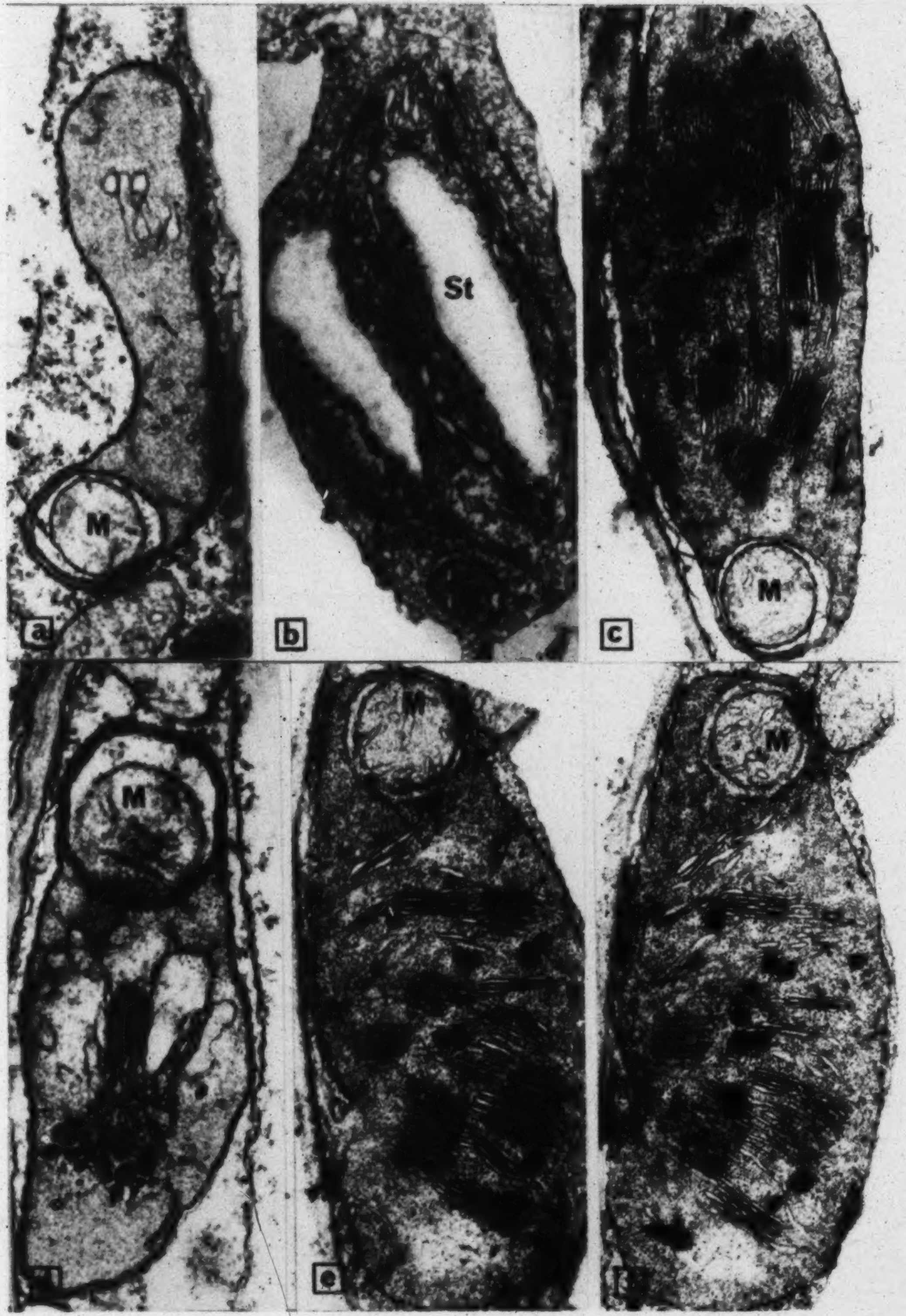
(a) 4 days at 1400 ft-c. A mitochondrion is seen inside the vacuole (arrow) of the chloroplast. X 42,500.

(b) 10 days in dark then 2 days at 1400 ft-c. The double membrane of the mitochondrion and of the vacuole are attached. X 24,500.

(c) 7 days in dark, then 10 days at 1400 ft-c. Mitochondrion inside vacuole of the chloroplast. X 28,000.

(d) 4 days at 1400 ft-c. Part of the mitochondrion is in close association with the stroma of the chloroplast. X 40,800.

(e) and (f) 7 days in dark and then 10 days at 1400 ft-c. Serial sections of a plastid in which the mitochondrion completely occupies the vacuole. Also mitochondrial cristae are seen in contact with the stroma. X 35,000.


M
a
St
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M
c
M
d
M
e
M
f

serial sections of a plastid in which the double membrane of the vacuole is not apparent and the space is almost completely occupied by the mitochondrion. Also, the mitochondrial cristae appear to be in contact with stroma (Fig. 23e, arrow).

Another feature observed frequently in the developing mutant barley plastids was the presence of cytoplasmic material other than mitochondria within their vacuoles (Fig. 24a and f). In studying an albino mutant of *Helianthus annuus*, Walles (1965) observed vacuoles containing cytoplasmic material and mitochondria in degenerating plastids. The heavily stained matter in Fig. 24b does not resemble cytoplasmic material and Fig. 24g shows a group of membranes in the vacuole of a plastid. The frequency of plastids with vacuoles was greater for the younger seedlings of the mutant than the older ones. In fact, this is another abnormality in development of plastids of the mutant seedlings which disappears when their chloroplasts develop normal grana and stroma lamellae.

One question that may be raised relevant to these observations is how did mitochondria and other cytoplasmic material get into the chloroplast? The sequence of events leading to vacuole formation and entrapping of the mitochondria by the chloroplasts may have occurred as follows. In early development the juvenile plastids of the mutant barley were deformed. A constriction appeared near the middle of some plastids giving them a dumbbell shape (Fig.

FIGURE 24. Plastids of the mutant barley depicting an endocytotic-like process.

Six days at 1400 ft-c.

(a) and (b) The double membraned vacuole (Va) of plastids contains cytoplasmic-like material (a and b) or unidentifiable material (b) which has a double membrane and is heavily stained with osmium. The arrow indicates joining of the double membrane of the vacuole and of the unidentifiable material. (a) X 44,600. (b) X 34,000.

(c) A plastid showing formation of a food cup from pseudopodia-like structures formed by the plastid envelope. The food cup contains some cytoplasmic material. X 40,600.

Four days at 600 ft-c.

(f) The vacuole of the plastid contains an unidentifiable material. X 36,700.

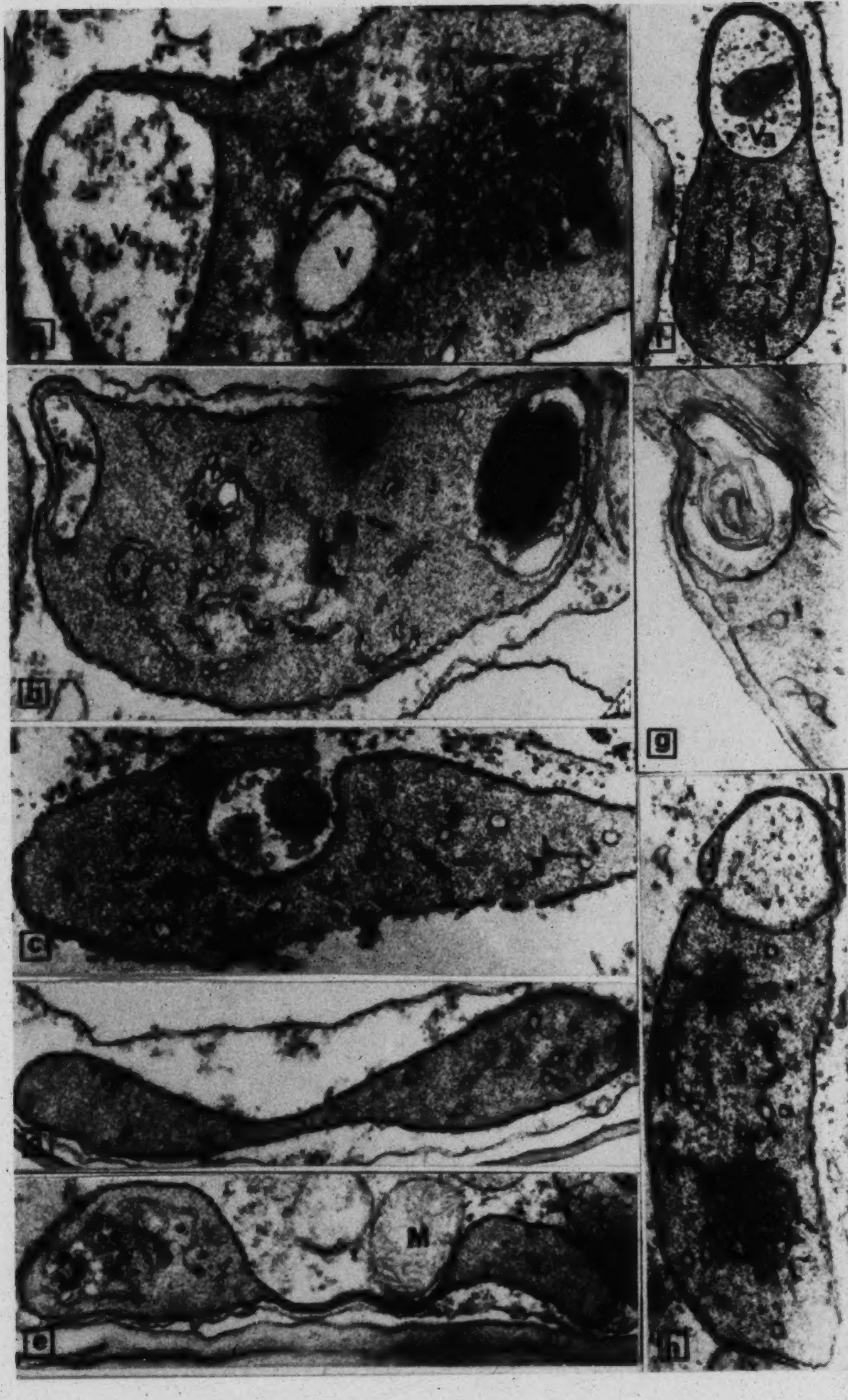
Four days at 1400 ft-c.

(d) A dumbbell-shaped plastid. X 42,500.

(e) A mitochondria is in close contact with slender part of a dumbbell plastid. X 42,500.

(g) The vacuole of a plastid containing a group of membranes. X 32,500.

(h) A plastid depicting possible formation of a vacuole. The tapered tail of the plastid is seen approaching the parent body. X 43,300.


V
V
a
Va
f
b
g
c
e
M
h

24d), while in others the middle part was long and slender (Fig. 24e). The half of the plastid which was smaller and devoid of lamellar material may have narrowed to form a tapering tail (Fig. 21g and 24h). Many chloroplasts with such structures were seen and the length of the tails varied in different plastids. During chloroplast breakdown in the trumpet of Daffodils, tail-like protruberances were observed by Nichols *et al.* (1967), which lengthened, turned back and fused with the parent body to form a vacuole.

In Fig. 21g and 24e mitochondria are seen in close contact with the slender part of chloroplasts. In Fig. 24h the tapered tail of the plastid is recurved and approaches another edge of the chloroplast forming a cavity in which some cytoplasmic material is visible. It is envisaged that a mitochondrion could be impounded in a like manner. The process of vacuolization which is observed in the plastids closely resembles the description of pinocytosis reported by Fawcett (1965) for endothelial cells in capillaries. In Fig. 24c the chloroplast membrane has formed a pseudopodia-like structure giving rise to a food cup (Edward, 1925) containing cytoplasmic material. The enfolded stroma resembles the folds of cytoplasm formed in amoeba during endocytosis. The occurrence of this process only in the undeveloped abnormal chloroplasts may be associated with the extreme deformability of their membranes.

Wildman *et al.* (1962) observed the dynamic behavior of chloroplasts in the living cells of spinach using cinephotomicrography. In living cells the outline of chloroplasts was constantly changing its shape. Chloroplast jackets were in continuous motion and long protuberances from them extended into the cytoplasm. They noted that protuberances segmented from time to time into bodies which were indistinguishable from mitochondria and that occasionally mitochondria united with chloroplasts losing their identity. Vesk *et al.* (1965) using the electron microscope also observed a high degree of deformability of the chloroplast envelop and frequent occurrence of mitochondria inside the mature chloroplasts in maize, barley and particularly in manganese-deficient spinach. According to them some of their electron micrographs suggested that mitochondria may be derived from chloroplasts, while in other electron micrographs it appeared that chloroplasts flowed around mitochondria thus enclosing the latter.

Although the electron microscope provides high resolution static pictures, they are only of limited value in elucidating the dynamic behavior of organelles within the living cell. In part this is due to the fact that during the preparation of tissue for electron microscopy the characteristics of the structures might be altered from their native state. While the dynamic behavior of organelles within a cell can be noted under phase contrast microscopy its resolution is limited.

One can only speculate about the significance of the appearance of mitochondria and other cytoplasmic material entrapped within the developing plastids. The electron micrographs shown here may depict a means by which chloroplasts and mitochondria could establish a symbiotic relationship. On the other hand, if plastids can indeed ingest mitochondria and other cytoplasmic material, it might represent further evidence for the semi-autonomous nature of chloroplasts.

There are two plausible explanations for the phenomenon described here. One is that some chloroplasts of the mutant are capable of engulfing cell organelles and other cytoplasmic components by means of an endocytotic-like process (endoplastosis), leading to the presence of mitochondria within vacuoles of chloroplasts. On the other hand, some of the micrographs may simply represent cross-sections of mitochondria appressed within an open-ended invagination of a chloroplast. Whether either or both of these alternatives are partially true, may be resolved eventually by obtaining complete sets of serial sections of whole chloroplasts.

III. Microtubule-like Structures of Chloroplasts

During the investigation of development of chloroplasts in greening of etiolated seedlings, two types of quasi-crystalline array (Fig. 25 and 26) were observed frequently within the stroma of chloroplasts, which may be different orientations of the same structure. These structures were located between the developing lamellae. Fig. 25a to f shows several clusters of electron-dense particles arranged hexagonally between the lamellae. Under high magnification the particles of the crystalline array appeared like a cross section of a thin tubule (Fig. 25b and c). Except for their smaller size the particles showed a marked resemblance to the widely occurring cytoplasmic microtubules (Ledbetter and Porter, 1963; Porter, 1966). Like microtubules these particles have a dense wall and an inner transparent core. In addition, they are also surrounded by a zone of low electron density. However, the micrographs do not reveal whether the wall of the tubule is made by close association of subunits as is observed in negatively stained preparations of plant cytoplasmic tubules (Porter, 1966). Some of these particles do not show electron-transparent cores and look opaque throughout (Fig. 25f). This could be due to the oblique sectioning of a thin tubule. The number and arrangement of particles in a cluster is variable. Fig. 25b,c,d,e and Fig. 26e show a few of the typical clusters.

FIGURE 25. Electron micrographs showing quasi-crystalline arrays of particles within the stroma of chloroplasts.

(a) A plastid showing groups of particles. Low magnification. X 32,400.

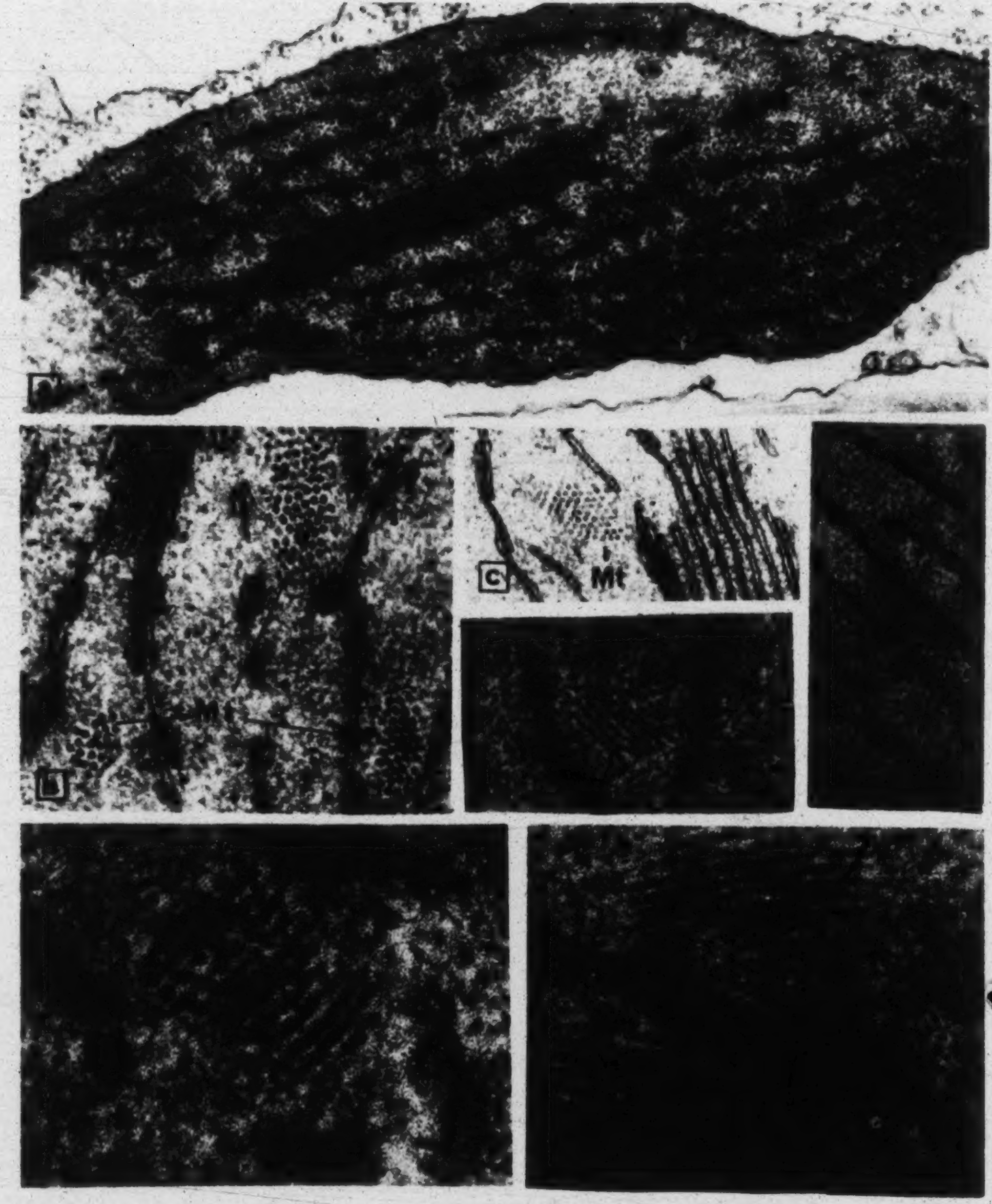
(b) and (c) High magnification. The particles have a dense wall and an inner electron-transparent core. They are arranged hexagonally. A zone of low electron density surrounding each particle is visible. X 58,800.

(d) and (f) A section showing relationship between two types of array of particles. (d) X 58,800.

(f) X 149,200.

(e) Similar to a. X 32,400.

(g) Longitudinal section of particles. X 132,800.


a
b
Mt
c
Mt

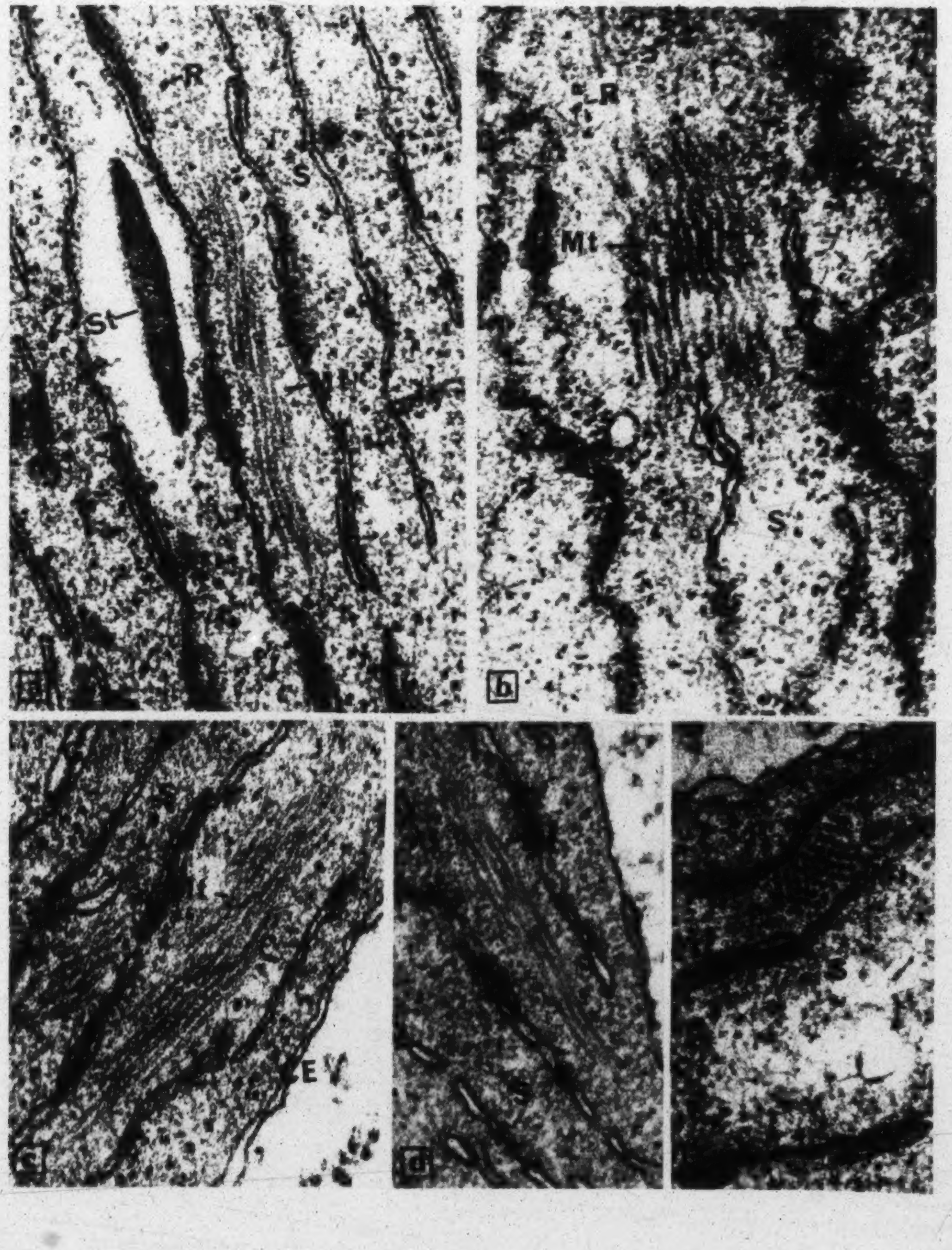
Other electron micrographs (Fig. 26 a to d) showed a parallel array which appeared to be bundles of fibrils which are more electron dense at the sides than in the center (Fig. 26c and d). These bundles appeared to be spiraled along their length. Their length appeared to be variable and they were not always straight. These structures do not show evidence of branching and blebbing like cytoplasmic microtubules. As observed in the cross sectioned tubules, these fibrils were also surrounded by a zone of low electron density. The structures appeared to be cylindrical and the crystalline aggregates of particles are probably cross-sections of these long tubules. The probable identity of the two types of array is demonstrated by photographs of intermediate appearance (Fig. 25d and f) which probably represent other orientations of the crystals in the sections.

Due to certain similarities between these fine-tubules and cytoplasmic microtubules some of the experiments suggested by Benke and Forer (1967) were employed to compare their characteristics. Although in their general morphology, all microtubules are very much alike, Benke and Forer (1967) have shown that various experimental treatments can demonstrate whether different microtubules have similar characteristics. To see whether the bundles of fine tubules have similar properties to cytoplasmic microtubules the fixation properties and cold sensitivity of chloroplast fine tubules were tested. For

FIGURE 26. Electron micrographs showing parallel array of bundles of fibrils within the stroma of chloroplasts.

(a, b, c and e) Fixed in $KMnO_4$. The fibrils appear cylindrical and are surrounded by an area of low electron density. In longitudinal section (a, b and c). (a, b) X 102,100. (c) X 116,400. A cross section (e). X 116,400.

(d) A section from cold treated tissue. Glutaraldehyde - OsO_4. X 88,200.


R
S
St
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Mt
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EV
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L

cytoplasmic microtubules the fixation by $KMnO_4$ and OsO_4 was inadequate and their preservation required glutaraldehyde (Ledbetter and Porter, 1963). In addition, according to Porter and co-workers (1965, 1967) and Inouè (1964), cold treatment effectively depolymerizes these tubules.

In this study it was found that the fine tubules of chloroplasts were well preserved in tissue which was fixed with 2% $KMnO_4$ (Fig. 26a,b) showing that they did not require special fixation and were therefore unlike cytoplasmic microtubules in this respect.

The fine tubules were not found to be sensitive to cold treatment since they were present in leaves or intact seedlings kept at 4°C for 1 to 3 hr before fixation at 4°C. Fig. 26d shows these structures from cold treated tissue. Thus the results suggest that by this criterion they are also not similar to cytoplasmic microtubules.

The fine tubules were observed most frequently in chloroplasts containing single parallel lamellae. It is possible, however, that they are also prevalent in the more developed chloroplasts but due to the presence of more lamellar material they were not observed.

Different kinds of inclusions have been reported in the chloroplasts of higher plants and algae (Hyde *et al.*, 1963; Gunning, 1965b; Gunning *et al.*, 1968; Bartels and Weier, 1967; Picket-Heaps, 1968; Sprey, 1968). The fine tubules observed in the present investigation have been previously reported in wheat (Bartels and Weier, 1967) and barley (Sprey, 1968; Henningsen and Boynton, 1970). Sprey (1968) considered their function in plastid division and in the formation of thylakoids. Whereas Henningsen and Boynton (1970) related them with stroma centers of oat and bean chloroplasts (Gunning *et al.*, 1968). 'Stroma centers' which are fibrillar spherulites have been observed in OsO_4-glutaraldehyde fixed material from oat and according to Gunning *et al.* (1968) they are aggregates of fraction I protein. In bean their formation was induced in leaves dehydrated by plasmolysis, wilting or high speed centrifugation. A comparison of the electron micrographs of fine tubules from the normal and mutant barley with stroma centers of oat and bean shows that the morphology and the occurrence of the two are quite different. The fibrils of the stroma centers are smaller in size and are not surrounded by electron transparent zones. Moreover, they are present in mature chloroplasts and together the small bundles occupy as much as 1/3 to 1/2 of the area of chloroplasts (Gunning *et al.*, 1968).

The structures observed in the barley chloroplasts are similar to the rod-like structures and crystalline aggregates of particles from etioplasts of wheat (Bartels and Weier, 1967). Microtubule-like structures have been reported in the chloroplasts of *Chara fibrosa*, *Nitella* and *Volvox* (Picket-Heaps, 1968). In these three algae difference in size of the microtubule-like structures were noted. In *Chara* the tubules had variable diameter and were in groups of from 1 to 20. The occurrence of these structures in chloroplasts of higher plants and in algae suggests that their presence may have a more general significance.

The characteristics of the fine tubules in cross section resembled those seen in the chloroplasts of virus infected *Beta vulgaris* (Cronshaw *et al.*, 1966). In sections of virus infected leaves particles present in chloroplasts looked significantly different than the virus particles present in the surrounding cytoplasm. Although the authors reported that their presence was due to virus infection this has not been proven unequivocally. So far as the barley varieties are concerned there is no basis for concluding that the observed fine tubules are due to virus infection.

To hypothesize a function for these microtubule-like structures on the basis of electron-micrographs is difficult. However, their occurrence within the stroma of chloroplasts might in some way account for the high degree of order exhibited by the lamellae during the course of development of the latter. Because of this possibility it is of considerable interest to determine the frequency of occurrence and orientation of the microtubule-like structures relative to the lamellae within the chloroplasts. If the tubules which are about 110 Å in diameter are arranged parallel to the surface of the sections, which are about 600 Å thick, the possibility of observing them would depend upon their number, their orientation and distribution in the section. On the other hand, when they are oriented perpendicular to the sections their detection is more likely. In any event, it is extremely difficult to obtain an accurate estimate of the amount and function of the microtubule-like structures.

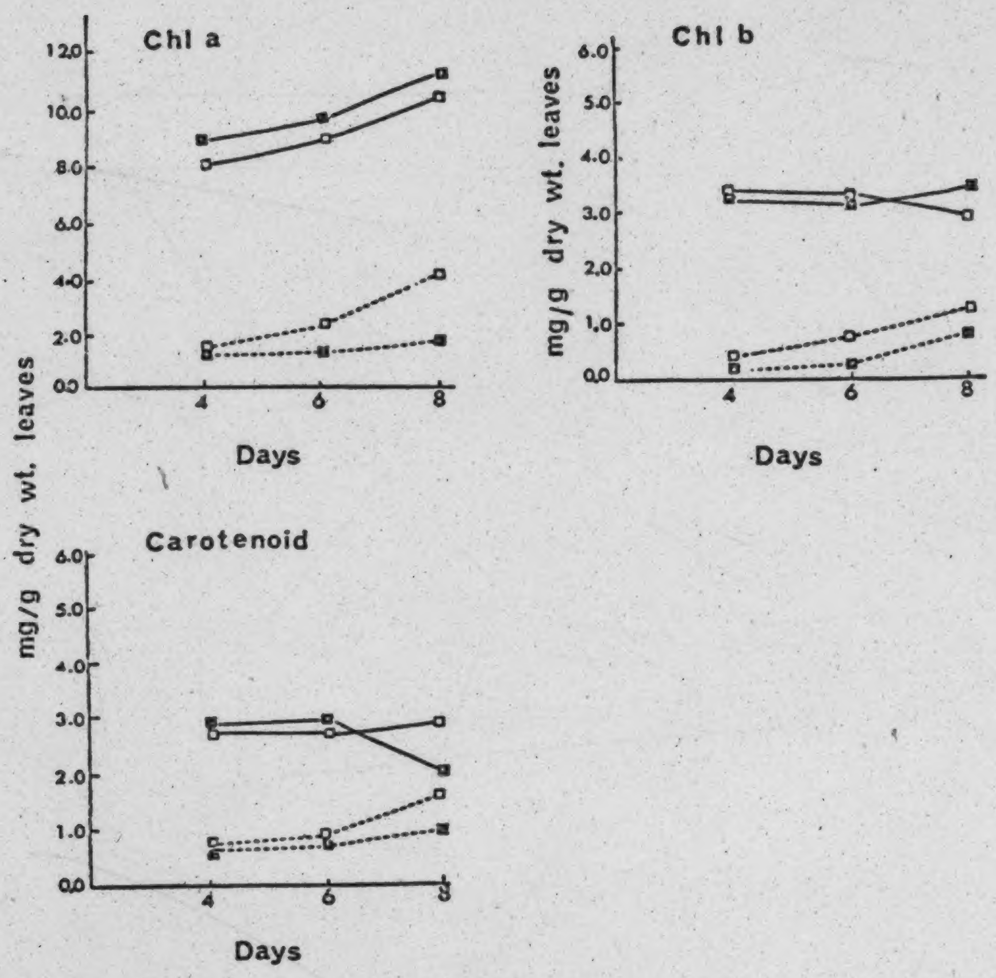


Fig. 27. Concentration of chlorophyll a, chlorophyll b and carotenoids in seedlings of Gateway barley and its mutant at 4, 6 and 8 days. Solid lines, normal; broken lines, mutant, 1400 ft-c at 22°C ■, 600 ft-c at 20°C □.

IV. Pigments and Proteins

Pigments.

Pigment levels of the two lines have previously been compared by Maclachlan and Zalik (1963) and Miller and Zalik (1965) but their data could not be applied directly to the present work due to differences in growing conditions. Thus estimates of pigments had to be made in conjunction with these studies, but they were done in a cursory manner. Fig. 27 shows that the amount of chl a, chl b and carotenoids in the normal seedlings was greater than in the mutant under both light intensities at 4, 6 and 8 days. Although normal seedlings grown under 1400 ft-c contained slightly more chlorophyll a than those grown under 600 ft-c the reverse was noted in the mutant. By 8 days the mutant accumulated only 40% and 20% of the amounts present in the normal seedlings grown at 600 ft-c and 1400 ft-c respectively. Studies by Maclachlan and Zalik (1963) showed that the mutant contained the same complement of chlorophyll and carotenoid pigments as the normal, but they were present in lower amounts in the mutant. Under different light intensity and temperature conditions the amount of pigment in the mutant was always lower than normal (Miller and Zalik, 1965). It was found that at 14°C the mutant accumulated less pigments at the higher light intensity.

Proteins

Soluble leaf proteins.

Although electrophoresis of stroma proteins of chloroplasts was conducted initially the results were not reproducible. This may have been due to variability in the amounts of soluble proteins lost with different chloroplast preparations. Therefore, the soluble leaf proteins of the two barley lines were compared.

To obtain good resolution of the proteins various conditions of electrophoresis were investigated. Gels containing three different concentrations of acrylamide 7.5%, 9.5% and 11.5% were used. The best separation was achieved with 9.5% gels. The separation of the protein was also affected by the duration of run and by the length of gel. Tubes 12 cm in length gave unsatisfactory separation because high mobility protein bands were concentrated towards the end of the gel and were not resolved. If the long gel tubes were run only for 4 cm the separation achieved was better, hence tubes of 6 cm length were used and these provided good resolution. A series of concentrations of the leaf extract was applied to determine a nearly optimal amount for use in electrophoresis.

A current of 3 mA/tube was found more suitable for separation than 4 or 5 mA/tube. Although photopolymerized gels have been recommended for the separation of leaf proteins (Steward and Barber, 1964) no significant differences in their separation were observed in the present study when chemically polymerized and photopolymerized gels were used.

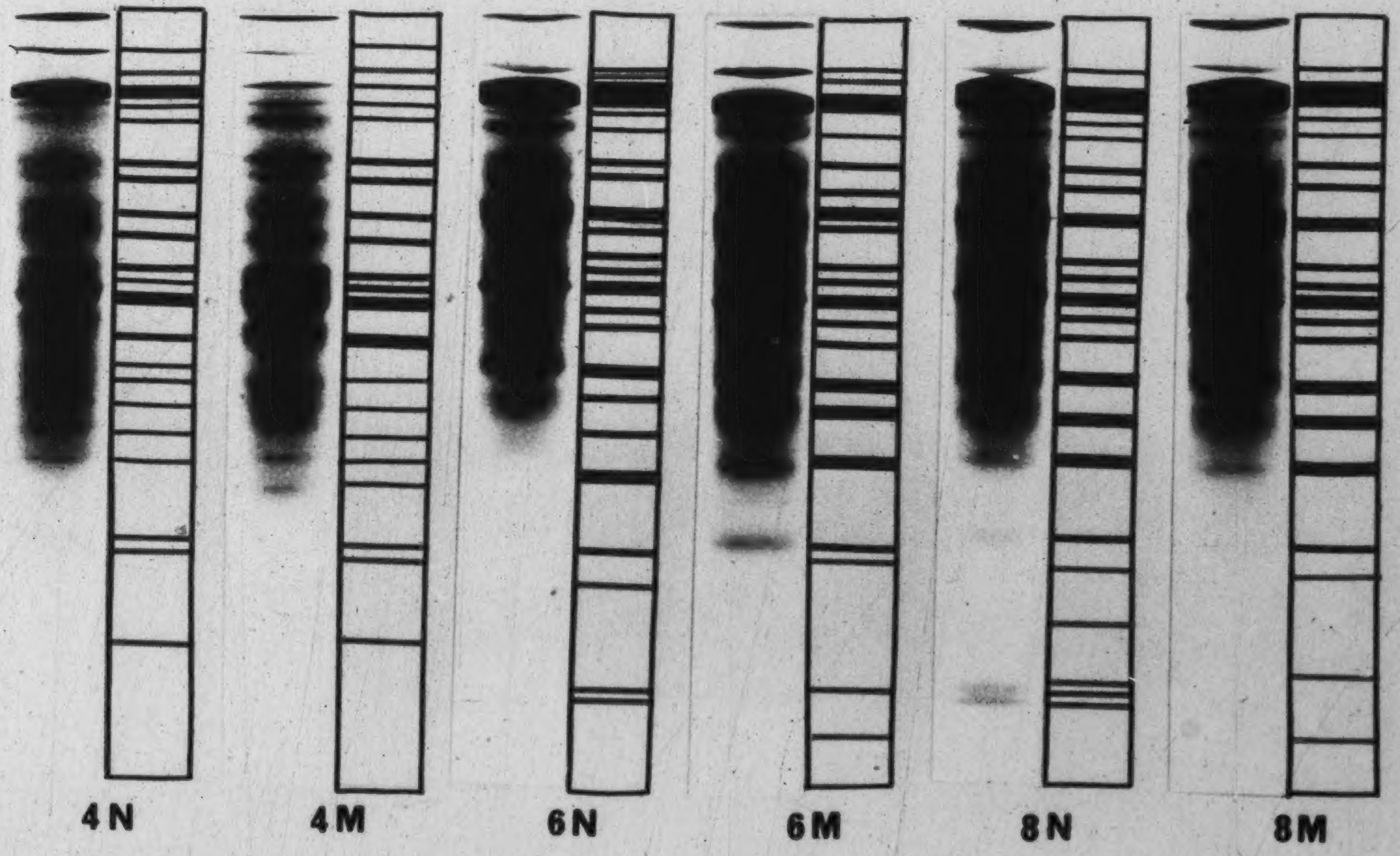
Typical electrophoretic patterns of soluble leaf proteins from various ages of Gateway barley and its mutant are shown in Fig. 28. Twenty-one discrete bands of soluble proteins were resolved in 4-day-old seedlings. A broad intense band of low mobility was apparent at all stages except in the 4-day-old mutant. It appeared to be the most abundant soluble protein of the leaf. The main difference between the normal and mutant seedlings was the quantity of this protein in the 4-day-old mutant. It increased in amount with age and by 8 days was similar in intensity in both lines. Some increase in the amount of this protein also occurred in normal barley with age.

Apart from the major band the intensity of some other bands also increased in older seedlings of both lines. Although the bands were well resolved when the gels were viewed on a light box a diffuse background which was barely apparent was magnified by photography.

As was mentioned earlier in this section, the results obtained upon electrophoresis of stroma proteins were not reproducible, therefore, the total soluble leaf proteins were compared. However, in the studies in which stroma proteins were electrophoresed a major protein of low mobility was always noted in the normal which corresponded in its position on the gel to that of the major leaf

FIGURE 28. Gel patterns of soluble leaf proteins of Gateway barley (N) and its mutant (M) grown for 4, 6 and 8 days under continuous illumination of 600 ft-c at 20°C.

Leaf extract containing 75 to 100 µg of protein was applied to gels (see methods) and electrophoresed at 4°C. All runs were done in duplicate and repeated 5 times with fresh leaf material.


4N
4M
6N
6M
8N
8M

protein when the soluble leaf proteins were compared. These results suggest that the major soluble leaf protein belongs to chloroplasts.

Studying the soluble leaf proteins of rice, Mendiola and Akazawa (1964) obtained 7 bands on starch gel electrophoresis, and Wrigley *et al.* (1966) noted 17-18 protein bands on acrylamide gels of wheat leaf proteins. Mendiola and Akazawa (1964) identified the major band of low mobility as fraction I protein. Thornber *et al.* (1966) also identified the major protein band of low mobility from spinach chloroplasts as fraction I, in agreement with Lyttleton and Ts'o (1958) who considered it to be a major component of the stroma.

Kannangara (1969) isolated fraction I protein from barley leaves and found that RuDP carboxylase activity was exclusively associated with this fraction. A method for isolating this enzyme in relatively pure form was developed by Kleinkopf *et al.* (1970).

Since the soluble leaf proteins of barley in this study were electrophoresed by a method like that employed by Wrigley *et al.* (1966) and Thornber *et al.* (1966) it is probable that the major band of low mobility observed was likewise fraction I protein. As mentioned above it is possible that in this case it was also derived from the stroma. Further support for the latter may be the finding that the mutant contained very little of this band at 4 days when the plastids were poorly developed but the level increased as the plastids developed further (Fig. 22 and 28).

Chloroplast lamellar proteins.

Electrophoresis for 2 hr of SDS-solubilized lamellae from normal barley chloroplasts separated into three distinct green coloured components designated as I, II and III (Fig. 29). On staining the gels with amido black or coomassie blue components I and II stained for proteins. Also stained zones in which the proteins were not completely resolved appeared between the three coloured bands. From these results it appeared that components I and II consisted of proteins as well as pigments, the stained regions contained only proteins and component III was a mixture of free pigments. When electrophoresis was carried out for 4 hr resolution of the stained zones into several proteins occurred. This also affected the pigment-protein bands I and II. During the longer electrophoresis a gradual loss of pigments from these components was noted and at the end of the run only band I still contained pigment.

Fig. 29 shows the results obtained after a short run electrophoresis of solubilized chloroplast lamellae from normal and mutant barley grown under 600 ft-c and 1400 ft-c at various stages of development. The pattern of the pigment components in the normal barley did not change with age and was unaffected by light intensity. In the mutant, pigment bands I and II were totally absent at 4-days and

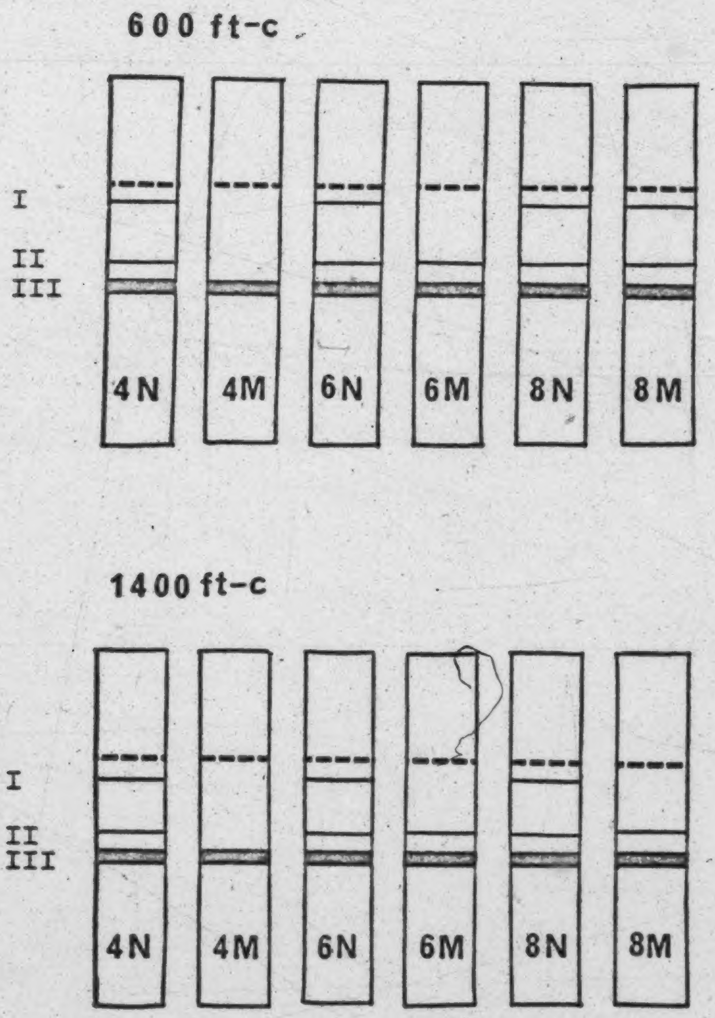

600 ft-c
I
II
III
4N
4M
6N
6M
8N
8M
1400 ft-c
I
II
III
4N
4M
6N
6M
8N
8M

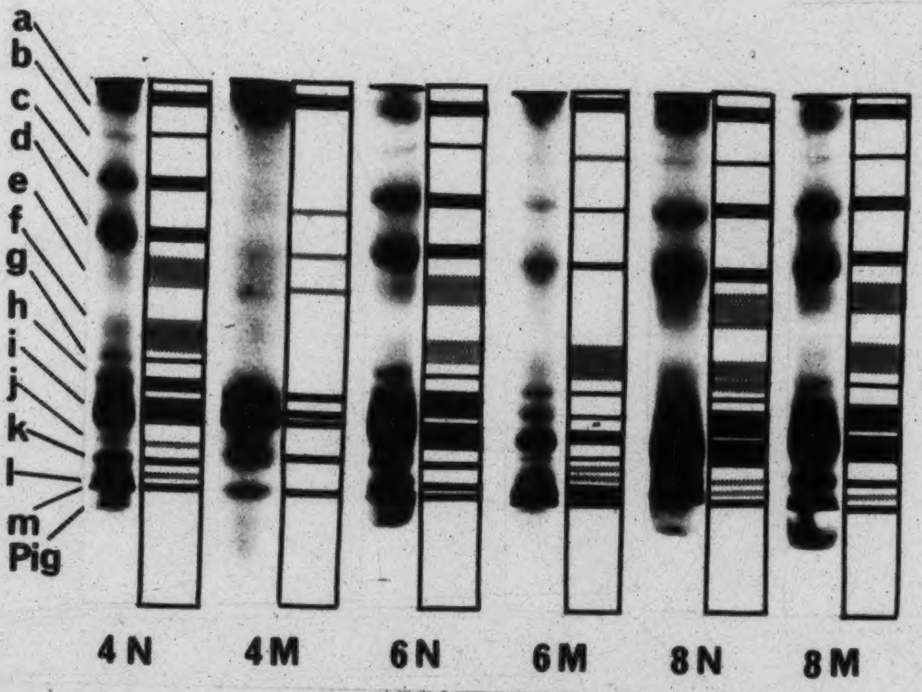
Fig. 29. Diagrammatic representation of components with pigment separated on short run electrophoresis of SDS solubilized lamellae of Gateway barley (N) and its mutant (M) grown for 4, 6 and 8 days under continuous illumination of 600 ft-c at 20°C (upper) and 1400 ft-c at 22°C (lower). Extract containing 50-100 μg protein was applied to gels prepared as given in methods. Electrophoresis was performed at 24±2°C for approximately 2 hr. All runs were in duplicate and repeated at least 5 times.

only a pale yellow colour was present at the solvent front which corresponded to component III in normal barley. At 6 days component II appeared in the gel but component I was absent. In stained gels of the mutant, pigment component II stained for protein and a protein band appeared at the same position as component I of the normal. By 8 days component I also appeared in the mutant and the gel pattern of the two barley lines was similar when grown at 600 ft-c and 20°C. The mutant seedlings grown at 1400 ft-c and 22°C lacked component I and II at 4 days and component I at 6 and 8 days.

Fig. 30 shows the gel pattern obtained after electrophoresis of the lamellar proteins for 4 hr. Before staining the gels, a green band was present at the solvent front and at the site of protein band b of the normal. This corresponded to green band I of the short run gels (Fig. 29) and was also observed in 8-day-old mutant seedlings grown under 600 ft-c. The solvent front of the 4- and 6-day mutant seedlings was pale yellow to yellow green. Upon staining the gels a number of protein bands were detected in both lines.

Examination of the electrophoretic pattern shows that the major differences between the lamellar proteins of the normal and mutant barley under both growth conditions were observed in the 4-day-old seedlings (Fig. 30). At the lower light intensity the mutant had fewer bands at this age. Whereas 13 bands (a to m) were resolved in the normal,

FIGURE 30. Chloroplast lamellar proteins of Gateway barley (N) and its mutant (M) grown for 4, 6 and 8 days under continuous illumination of 600 ft-c at 20°C (upper) and 1400 ft-c at 22°C (lower). Extract containing 50 - 100 μg protein was applied to gels prepared as given in methods. Electrophoresis was performed at 24±2°C for approximately 4 hr. All runs were in duplicate and repeated at least 5 times.


a
b
c
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e
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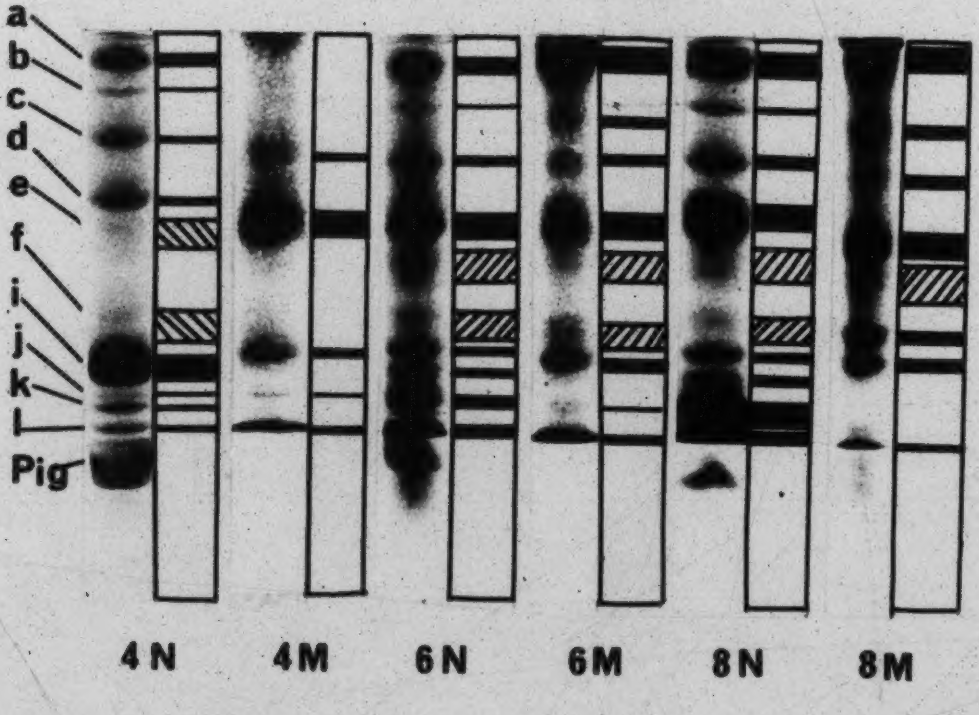
h
i
j
k
l
m
Pig
4N
4M
6N
6M
8N
8M


a
b
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e
f
i
j
k
l
Pig
4N
4M
6N
6M
8N
8M

the mutant had 3 faint bands and 6 intense bands. An increase in the number of bands had occurred by day 6 and except for band b the mutant was similar to the normal. Although lacking pigment, protein band b of the mutant corresponded in mobility to the pigment-protein band b of the normal. By 8-days no differences were observed between the lamellar proteins of the two lines grown under 600 ft-c at 20°C but the intensity of some protein bands in both the normal and mutant increased with age.

It can be seen that fewer lamellar proteins were resolved in both lines when they were grown at 1400 ft-c than at 600 ft-c (Fig. 30) and again at 4 days the mutant had fewer bands than the normal. At this age the mutant had 4 intense bands and 1 weak band compared to 10 in the normal. Although the mutant had more proteins by 6 and 8 days it still had fewer than the normal. The intensity of some bands in both lines increased with age.

Several workers have reported the separation of SDS or SDBS-solubilized lamellae from chloroplasts into two pigment-protein complexes when they were electrophoresed for periods less than 2 hr (Ogawa *et al.*, 1966, 1968; Thornber *et al.*, 1967a,b; Machold *et al.*, 1971 ; Herrmann and Meister, 1972). These authors claimed that on the basis of chl a/b ratios, pigment composition and absorption properties of the pigments present in complexes I and II it was possible to separate PSI from PSII. Two pigment-protein components I and II were noted on brief electrophoresis of the SDS-solubilized lamellae of barley. These corresponded in their positions to the chlorophyll-protein complexes I and II reported in spinach (Ogawa *et al.*, 1966), *Beta vulgaris*, *Nicotina tabacum*, *Avena sativa* (Thornber *et al.*, 1967a), *Vicia faba* and *Chlorella pyrenoidosa* (Machold *et al.*, 1971) and *Anabaena variabilis*, *Porphyria* (Ogawa *et al.*, 1968). However, the fading of the pigment components and resolution of the lamellar proteins into 13 discrete bands upon longer electrophoresis (Fig. 30) suggests that the findings of Ogawa *et al.* (1966) and Thornber *et al.* (1967a) may be equivocal. Machold (1971a) noted 17 bands from *Phaseolus vulgaris* and reported that two bands with chlorophyll were chlorophyll-protein complexes like those of Thornber *et al.* (1967a). In *Antirrhinum majus* 15 bands were reported by Herrmann and Meister (1972) who claimed that 7 bands with pigment were chlorophyll-protein complexes which were visible only during the first 90 min of electrophoresis after which time the green colour vanished gradually. Remy (1971) reported that the electrophoretic patterns of lamellar proteins from spinach, wheat and barley were similar and of the 10 proteins resolved three were associated with pigments.

Herrmann (1971b) has reported that the plastom mutant of an albina of *Antirrhinum majus* lacked pigment-protein complex I and some other proteins. The mutant was capable of reducing the Hill oxidants DPIP and ferricyanide, however it was impaired in photosystem I (Herrmann, 1971b).

Gregory *et al.* (1971) also reported that Bishop mutant 8 of *Scenedesmus obliquus* lacks a functional photoreaction I and pigment-protein complex I. He concluded that lack of a functional photosystem I in the mutant was due to the absence of chlorophyll-protein complex I.

As stated earlier the virescent barley mutant under study lacked chlorophyll-protein complexes I and II at 4 days. Nevertheless it was found from other studies in our laboratory (Horak and Zalik, 1973) that even at this early stage the mutant had both PSI and PSII activity. It is possible that true chlorophyll-protein complexes dissociate in the course of electrophoresis, if so the bond between pigment and protein is very unstable. From the present studies it seems that the pigment-protein bond in complex I is more stable than that of complex II. This is in agreement with earlier findings reported by Bailey and Kreutz (1969).

Thornber *et al.* (1967a) noted several other proteins in addition to chlorophyll-protein complexes, however, they attributed the pigment-free proteins to contamination from the stroma. In the present work, lamellae were extracted several times with buffer and water prior to solubilizing their proteins, therefore the proteins were assumed to represent the non-stroma proteins of the chloroplasts. Furthermore the studies by Machold, Herrmann and Meister and by Remy showed the presence of many proteins without pigment in the lamellae.

The electrophoretic patterns of the soluble and lamellar proteins show that plastids lacked several proteins early in development but were able to form them as growth progressed. A comparison of the soluble and lamellar proteins and pigment levels of Gateway and its mutant at different ages with the corresponding states of development of their chloroplasts revealed a relationship between chloroplast morphology and the proteins and pigments present.

Previous studies on the mutant have shown that on greening the mutant was initially exceedingly low in chlorophyll compared to the normal and that it accumulated chlorophyll at a lower rate (Maclachlan and Zalik, 1963). Similarly in the present study it was found that the mutant at 4 days contained lower levels of pigments and furthermore the increase in pigments was correlated with the appearance of proteins at 6 and 8 days.

Sections from the seedlings used in the protein investigation were examined under the electron microscope. The electron micrographs and accompanying discussion appears on pages 76 - 98. Although plastids of the normal were well developed at 4 days those of the mutant were not and the latter contained lower amounts of pigments and fewer proteins compared to the normal. By 6 days the increase in the amount of pigments and the appearance of proteins was accompanied by an increase in lamellar material of the chloroplasts. By 8 days the electrophoretic patterns of the two barley lines were similar (Fig. 30) and the mutant contained plastids which appeared like those of normal seedlings grown at 600 ft-c and 20°C (Fig. 22). These results show that accumulation of one component may have influenced the synthesis of the others, thus affecting chloroplast morphology. The formation of pigments (chl a, b and carotenoids), soluble proteins and lamellar proteins of chloroplasts and their incorporation in the lamellae seemed to be synchronized suggesting that their synthesis have interdependent controls. The differences in the rate of development of the mutant when grown at 600 ft-c and 1400 ft-c supports this view. When grown at 1400 ft-c the development of the mutant in terms of the amount of pigment, the number of chloroplast proteins and plastid structure was delayed. Even at the 8-day stage it was not normal in any of these respects.

Although the barley mutant involves a single nuclear gene (Stephansen and Zalik, 1970; Walker *et al.*, 1963) which manifests itself by low pigment levels and poorly developed chloroplasts in the juvenile seedlings, with time these correct themselves (Maclachlan and Zalik, 1963; Miller and Zalik, 1965; Sane and Zalik, 1970). The major soluble protein as well as the lamellar proteins which were initially low or absent are restored with time (Fig. 29 and 30). Thus the action of this gene does not result in the total absence of any of the components so far investigated. Studying nuclear gene mutants of barley which had low RuDP carboxylase activity, von Wettstein *et* *al.* (1971) found that none of them lacked the ability to synthesize the enzyme.

The control and site of synthesis of RuDP carboxylase and membrane polypeptides have thus far been studied mainly with the use of specific antibiotic inhibitors (Criddle *et al.*, 1970; Hoober, 1970, 1972; Ireland and Bradbeer, 1971; Margulies, 1971). Rutner and Lane (1967) found that on treatment with SDS, RuDP carboxylase from spinach dissociated into two distinct subunits. Using a mutant strain of *Chlamydomonas reinhardi* and inhibitors of protein synthesis, Hoober (1970, 1972) concluded that both subunits of RuDP carboxylase as well as most membrane polypeptides were synthesized in the chloroplast, but a few membrane polypeptides were synthesized on cytoplasmic ribosomes. In contrast, Criddle *et al.* (1970) studying RuDP carboxylase synthesis in barley suggested that one subunit of the enzyme was synthesized in the cytoplasm and the other in the chloroplasts. Clarification of this problem requires further investigation. The use of suitable mutants from higher plants, like the viable virescent barley mutant discussed here, may be useful in elucidating the problem.

CONCLUSION

Miller and Zalik (1965) from studies on the virescent barley mutant concluded that the deficiency in chlorophyll synthesis was related to some defect in holochrome protein of the chloroplast. Other investigators using antibiotic inhibitors of protein synthesis have suggested that synthesis of chlorophyll was dependent on the synthesis of chloroplast proteins (Margulies, 1962; Bogorad and Jacobson, 1964). Also it has been shown that both chloroplast and cytoplasmic ribosomes are involved in the synthesis of chloroplast proteins (Eytan and Ohad, 1970; Hoober, 1970; Srivastava *et al.*, 1971).

Eytan and Ohad (1970, 1972) have investigated the control pattern involved in the formation of chloroplast lamellae in *Chlamydomonas reinhardi y-1* mutant. Their results suggested that a protein synthesized on cytoplasmic ribosomes was a prerequisite for the synthesis of chloroplast proteins and chlorophyll. On the other hand, they reported that light-dependent conversion of protochlorophyll to chlorophyll in turn acted as a control on the synthesis of some cytoplasmic protein. Studies by Hoober (1972, 1973) on the same algal mutant suggested that some precursor of chlorophyll regulated transcription of the m-RNA for a polypeptide synthesized on cytoplasmic ribosomes and its regulation was mediated by a protein synthesized within the chloroplast. These studies indicate that control mechanisms in chloroplast membrane formation are very complex and that proteins formed in the cytoplasm as well as in the chloroplasts are required to form functional chloroplast membranes. A similar but more complex regulation may be operating in biogenesis of chloroplasts in higher plants.

The findings from EM study on plastid development during greening of dark-grown seedlings of Gateway barley were similar to those reported for other higher plants. Microtubule-like structures within the stroma of chloroplasts were noted. From preliminary study these appeared to be different than cytoplasmic microtubules which have been described in the literature.

The mutation affected chloroplast development at the proplastid stage and there was a general lag in plastid development of the mutant. An apparent delay in formation of the lamellar system and a simultaneous delay in appearance of microtubule-like structures was also found. In addition to slower development of the chloroplasts some abnormalities were noted. Among these were large vesicles and different orientations of grana. Also a high degree of deformability of chloroplast envelopes leading to an endocytotic-like phenomena was observed. Due to the absence of crystalline prolamellar bodies it was inferred that etioplasts were deficient in holochrome-protein as well as protochlorophyllide. Upon illumination there was synthesis of more lamellar material which along with vesicles formed grana. Since stroma lamellae were formed later than grana lamellae it is probable that the proteins required for the two types of lamellae were different.

The synthesis of chloroplast proteins appears to depend upon the function of chloroplast and cytoplasmic ribosomes. From the electron micrographs it was determined that the mutant was deficient in plastid ribosomes. It is reasonable to assume that the deficiency might affect chloroplasts from the beginning of their development. If the formation of plastid ribosomes is coded by nuclear DNA then the delay in ribosomes formation in the virescent mutant might be a partial explanation for its lag in chloroplast development.

Young plastids of the mutant were deficient in pigments and lacked some of the proteins in comparison to the normal. During the course of development a gradual increase in pigments and appearance of proteins was accompanied by the structural normalization of the plastids. The number of proteins, pigment production and chloroplast structure were affected by light intensity. Sane and Zalik (1970) found that deficiency in chlorophyll production was not due to inadequacy of δ-amino levulinic acid, however, the mutant was less efficient in the synthesis of proteins. They suggested that the mutation had caused a partial inhibition in the synthesis of holochrome protein. In the present investigation of proteins in the developing mutant it was found that initially in young plastids some of the lamellar proteins were not detected. In the first few days of development Fraction I protein, with which RuDP carboxylase activity is exclusively associated, was also deficient in the mutant. Thus photosynthesis in it would be limited. It is probable that among the undetected proteins of the mutant there was a key protein which was affected primarily by the nuclear mutation and which might be a prerequisite for the formation of other lamellar proteins and pigments.

LITERATURE CITED

Akoyunoglou, G., J.H. Argyroudi-Akoyunoglou, A. Guiali, and C. Dassiou. 1970. On the relationship between ribulose diphosphate carboxylase and protochlorophyllide holochrome of *Phaseolus vulgaris*. *Plant Physiol*. 45: 443-446.

Akoyunoglou, G., and H.E. Siegelman. 1968. Protochlorophyllide resynthesis in dark grown bean leaves. *Plant Physiol*. 43: 66-68.

Anderson, I.C., and D.S. Robertson. 1960. Role of carotenoids in protecting chlorophyll from photodestruction. *Plant Physiol*. 35: 531-534.

Anderson, J.M., and N.K. Boardman. 1966. Fractionation of the photochemical systems of photosynthesis. I. Chlorophyll content and photochemical activities of particles isolated from spinach chloroplasts. *Biochim. Biophys. Acta*, 112: 403-421.

Anderson, L.E., C.B. Price, and R.C. Fuller. 1968. Molecular diversity of the ribulose-1,5-diphosphate carboxylase from photosynthetic microorganisms. *Science*, 161: 482-484.

Appelqvist, L.A., P.K. Stumpf, and D. von Wettstein. 1968*a*. Lipid synthesis and ultrastructure of isolated barley chloroplasts. *Plant Physiol*. 43: 163-187.

Appelqvist, L.A., J.E. Boynton, K.W. Henningsen, P.K. Stumpf, and D. von Wettstein. 1968*b*. Lipid biosynthesis in chloroplast mutants of barley. *J. Lipid Res*. 9: 513-524.

Arnon, D.L. 1959. Conversion of light into chemical energy in photosynthesis. *Nature*, 184: 10-21.

Argyroudi-Akoyunoglou, J.H., Z. Feleki and G. Akoyunoglou. 1971. Formation of two chlorophyll-protein complexes during greening of etiolated bean leaves. *Biochem. Biophys. Res. Commun*. 45: 606-614.

Bachmann, M.D., D.S. Robertson, C.C. Bowen, and I.C. Anderson. 1967. Chloroplast development in pigment-deficient mutants of maize. I. Structural anomalies in plastids of allelic mutants at W_3 locus. *J. Ultrastruct. Res.* 21: 41-60.

Bachmann, M.D., D.S. Robertson, and C.C. Bowen. 1969. Thylakoid anomalies in relation to grana structure in pigment-deficient mutant of *Zea mays*. *J. Ultrastruct. Res.* 28: 435-451.

Bailey, J.L., and W. Kreutz. 1969. Characterization of pigment-protein complexes related to photosystems I and II. *In* Progress in Photosynthetic Research. Vol. I. *Edited by* H. Metzner Tübingen. pp. 149-158.

Bartels, P.G., and T.E. Weier. 1967. Particle arrangement in proplastids of *Triticum vulgare* L. seedlings. *J. Cell Biol.* 33: 243-253.

Bell, P.R., and K. Mühlethaler. 1962. The fine structure of cells taking part in oogenesis in *Pteridium aquilinum*. *J. Ultrastruct. Res.* 7: 452-466.

Bell, P.R., and K. Mühlethaler. 1964. Evidence for the presence of deoxyribonucleic acid in the organelles of the egg cells of *Pteridium aquilinum*. *J. Mol. Biol.* 8: 853-862.

Benedict, C.R., and R.J. Kohel. 1968. Characteristics of a virescent cotton mutant. *Plant Physiol.* 43: 1611-1616.

Benedict, C.R. and R.J. Kohel. 1969. The synthesis of ribulose-1,5-diphosphate carboxylase and chlorophyll in virescent cotton leaves. *Plant Physiol.* 44: 621-622.

Benedict, C.R., and R.J. Kohel. 1970. Photosynthetic rate of a virescent mutant lacking chloroplast grana. *Plant Physiol.* 45: 519-521.

Benke, O., and A. Forer. 1967. Evidence for four classes of microtubules in individual cells. *J. Cell Sci.* 2: 169-192.

Bertagnolli, B.L., and M.J. Nadakavukaren. 1970. An ultrastructural study of pyrenoids from *Chlorella pyrenoidosa*. *J. Cell Sci.* 7: 623-630.

Biggins, J., and R.B. Park. 1965. Physical properties of spinach chloroplast lamellar proteins. *Plant Physiol.* 40: 1109-1115.

Bisalputra, T., and A.A. Bisalputra. 1967. Chloroplast and mitochondrial DNA in a brown algae *Egregia* *menziesii. J. Cell Biol.* 33: 511-520.

Bisalputra, T., and A.A. Bisalputra. 1969. The ultrastructure of chloroplasts of a brown alga *Sphacelaria* species. I. Plastid DNA configurations - the chloroplast genophore. *J. Ultrastruct. Res.* 29: 151-170.

Boardman, N.K. 1962. Studies on a protochlorophyll-protein complex. I. Purification and molecular-weight determination. *Biochim. Biophys. Acta,* 62: 63-79.

Boardman, N.K. and S.G. Wildman. 1962. Identification of proplastids by fluorescence microscopy and their isolation and purification. *Biochim. Biophys. Acta,* 59: 222-224.

Boasson, R., W.M. Laetsch, and I. Price. 1972. The etioplast-chloroplast transformation in tobacco; Correlation of ultrastructure, replication, and chlorophyll synthesis. *Amer. J. Bot.* 59: 217-223.

Bogorad, L. 1967. Biosynthesis and morphogenesis in plastids. *In* Biochemistry of Chloroplasts. Vol. II. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 615-631.

Bogorad, L., and A.B. Jacobson. 1964. Inhibition of greening of etiolated leaves by actinomycin D. *Biochem. Biophys. Res. Commun.* 14: 113-117.

Borisy, G.G., and E.W. Taylor. 1967. The mechanism of action of cholchicine. Cholchicine binding to sea urchin eggs and the mitotic apparatus. *J. Cell Biol.* 34: 535-548.

Boynton, J.E. 1966. Chlorophyll-deficient mutants in tomato requiring vitamin B_1. II. Abnormalities in chloroplast ultrastructure. *Hereditas,* 56: 238-254.

Boynton, J.E., and K.W. Henningsen. 1967. The physiology and chloroplast structure of mutants at loci controlling chlorophyll synthesis in barley. *Stud. Biophys.* 5: 85-88.

Branton, D., and R.B. Park. 1967. Subunits in chloroplast lamellae. *J. Ultrastruct. Res.* 19: 283-303.

Brown, F.A.M., and B.E.S. Gunning. 1966. Distribution of ribosome-like particles in *Avena* plastids.

In Biochemistry of Chloroplasts. Vol. I. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 365-373.

Cavalier-Smith, T. 1970. Electron microscopic evidence for chloroplast fusion in zygotes of *Chlamydomonas reinhardi*. *Nature*, 228: 333-335.

Chapman, D., and P.G. Fast. 1968. Studies of chlorophyll-lipid-water systems. *Science*, 160: 188-189.

Chollet, R., and D.J. Paolillo, Jr. 1972. Greening in a virescent mutant of maize. I. Pigment, ultrastructural, and gas exchange studies. *Z. Pflanzenphysiol*. 58: 30-44.

Chollet, R., and W.L. Ogren. 1972. Greening in a virescent mutant of maize. II. Enzyme studies. *Z. Pflanzenphysiol*. 68: 45-54.

Clewell, A.F., and G.H. Schmid. 1969. Chlorophyll-deficient *Lespedeza procumbens*. *Planta* (Berl.), 84: 166-173.

Cocking, E.C. 1970. Virus uptake, cell wall regeneration and virus multiplication in isolated plant protoplasts. *Int. Rev. Cytol*. 28: 89-124.

Criddle, R.S., B. Dau, G.E. Kleinkopf, and R.C. Huffaker. 1970. Differential synthesis of ribulose-diphosphate carboxylase subunits. *Biochem. Biophys. Res. Commun*. 41: 621-627.

Criddle, R.S., and L. Park. 1964. Isolation and properties of a structural protein from chloroplasts. *Biochem. Biophys. Res. Commun*. 17: 74-79.

Cronshaw, J., L. Hoefert, and K. Esau. 1966. Ultrastructural features of *Beta* leaves infected with beet yellows virus. *J. Cell Biol*. 31: 429-443.

Dale, J.E., and J.K. Heyes. 1970. A virescence mutant of *Phaseolus vulgaris*; growth, pigment and plastid characters. *New Phytol*. 69: 733-742.

Davis, B.J. 1964. Disc gel elctrophoresis. II. Method and application to human-serum proteins. *Ann. New York Acad. Sci*. 121: 404-427.

Drew, M.C., J. Seear, and A. McLaren. 1970. Entry of basic macromolecules in barley roots. *Amer. J. Bot*. 57: 837-843.

Edward, J.G. 1925. Formation of food-cups in *amoeba* induced by chemicals. *Biol. Bull.* 48: 236-239.

Emerson, R., and W. Arnold. 1932. The photochemical reaction in photosynthesis. *J. Gen. Physiol.* 16: 191-205.

Evan, L.V. 1966. Distribution of pyrenoids among some brown algae. *J. Cell Sci.* 1: 449-454.

Eytan, G., and I. Ohad. 1970. Biogenesis of chloroplast membranes. VI. Cooperation between cytoplasmic and chloroplast ribosomes in the synthesis of photosynthetic lamellar proteins during the greening process in a mutant of *Chlamydomonas reinhardi*. *J. Biol. Chem.* 245: 4297-4307.

Eytan, G., and I. Ohad. 1972. Biogenesis of chloroplast membranes. VIII. Modulation of chloroplast lamellae composition and function induced by discontinuous illumination and inhibition of ribonucleic acid and protein synthesis during greening of *Chlamydomonas reinhardi y-1* mutant cells. *J. Biol. Chem.* 247: 122-129.

Falk, H. 1969. Rough thylakoid: Polysomes attached to chloroplast membranes. *J. Cell Biol.* 42: 582-587.

Faludi-Daniel, A., L. Fridvalszky, and I. Gyurjan. 1966. Pigment composition and plastid structure in leaves of carotenoid mutants of maize. *Planta*, 78: 184-195.

Fawcett, D.W. 1965. Surface specializations of absorbing cells. *J. Histochem. Cytochem.* 13: 75-91.

Fillipovich, I.I., A.M. Tongur, B.A. Alina, and A.I. Oparin. 1970. Structural organization of the protein synthesizing system of chloroplasts. *Biokhimiya*, 35: 247-256.

Frey-Wyssling, A., and E. Steinmann. 1953. Über den Feinbau der Chlorophyllkörner. *Vierteljahressch. Naturf. Ges. Zürich*, 98: 20-28.

Gassman, M., S. Granick, and D. Mauzerall. 1968. A rapid spectral change in etiolated red kidney bean leaves following phototransformation of protochlorophyllide. *Biochem. Biophys. Res. Commun.* 32: 295-300.

Gibbs, S.P. 1962. The ultrastructure of the pyrenoids of green algae. *J. Ultrastruct. Res.* 7: 262-272.

Gibbs, S.P. 1968. Autoradiographic evidence of the *in situ* synthesis of chloroplast and mitochondria RNA. *J. Cell Sci.* 3: 327-340.

Gibor, A. 1967. DNA synthesis in chloroplasts. *In* Biochemistry of Chloroplasts. Vol. II. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 321-328.

Gilies, K.L., and V. Safaris. 1971. On the survival and reproduction of chloroplasts outside the cell. *Cytobios*, 4: 61-74.

Goodchild, D.J., H.R. Highkin, and N.K. Boardman. 1966. The fine structure of chloroplasts in a barley mutant lacking chlorophyll b. *Exp. Cell Res.* 43: 684-688.

Goodenough, V.W., and R.P. Levine. 1969. Chloroplast ultrastructure in mutant strains of *Chlamydomonas reinhardi* lacking components of photosynthetic apparatus. *Plant Physiol.* 44: 990-1000.

Goodenough, V.W., and R.P. Levine. 1970. Chloroplast structure and function in *ac-20*, a mutant of *Chlamydomonas reinhardi*. III. Chloroplast ribosomes and membrane organization. *J. Cell Biol.* 44: 547-562.

Gracen, V.E., J.H. Hilliard, and S.H. West. 1972. Presence of peripheral reticulum in chloroplasts of Calvin cycle cells. *J. Ultrastruct. Res.* 38: 262-264.

Granick, S. 1951. Biosynthesis of chlorophyll and related pigments. *Ann. Rev. Plant Physiol.* 2: 115-144.

de Greef, J., W.L. Butler, and T.F. Roth. 1971. Greening of etiolated leaves in far-red light. *Plant Physiol.* 47: 457-464.

Gregory, R.P.F., S. Raps, and W. Bertsch. 1971. Are specific chlorophyll-protein complexes required for photosynthesis. *Biochim. Biophys. Acta*, 234: 330-334.

Gunning, B.E.S. 1965*a*. The greening process in plastids. I. The structure of the prolamellar body. *Protoplasma*, 60: 111-130.

Gunning, B.E.S. 1965*b*. The fine structure of chloroplast stroma following aldehyde, osmium-tetraoxide fixation. *J. Cell Biol.* 24: 79-93.

Gunning, B.E.S., and M.P. Jagoe. 1967. The prolamellar Body. *In* Biochemistry of Chloroplasts. *Edited by* T.W. Goodwin, Academic Press, New York and London. pp. 655-676.

Gunning, B.E.S., M.W. Steer, and M.P. Cochrane. 1968. Occurrence, molecular structure and induced formation of the 'stroma centers' in plastids. *J. Cell Sci.* 3: 445-456.

Hadziyev, D., and S. Zalik. 1970. Amino acid incorporation by ribosomes and polyribosomes from wheat chloroplasts. *Biochem. J.* 116: 111-124.

Henningsen, K.W. 1967. An action spectrum for vesicle dispersal in bean plastids. *In* Biochemistry of Chloroplasts. Vol. II. *Edited by* T.W. Goodwin. Academic Press, London and New York. pp. 453-457.

Henningsen, K.W. 1970. Macromolecular physiology of the plastids. VI. Changes in membrane structure associated with shifts in the absorption maxima of chlorophyllous pigments. *J. Cell Sci.* 7: 587-621.

Henningsen, K.W., and J.E. Boynton. 1967. The physiology and ultrastructure of barley mutants at loci controlling the development of the lamellar systems in chloroplasts. *Stud. Biophys.* (Berl.), 5: 89-90.

Henningsen, K.W., and J.E. Boynton. 1969. Macromolecular physiology of plastids. VII. The effect of a brief illumination on plastids of dark-grown barley leaves. *J. Cell Sci.* 5: 757-793.

Henningsen, K.W., and J.E. Boynton. 1970. Macromolecular physiology of plastids. VII. Pigment and membrane formation in plastids of barley greening under low light intensity. *J. Cell Biol.* 44: 290-304.

Herrmann, F. 1971*a*. Genetic control of pigment-protein complexes I and Ia of the plastid mutant *en: alba-1* of *Antirrhinum majus* L. *FEBS Letters*, 19: 267-269.

Herrmann, F. 1971*b*. Structur und Function der genetischen

Information in den Plastiden. II. Untersuchung der Photosynthesedefekten Plastommutante *abla-1* von *Antirrhinum majus* L. *Photosynthetica*, 5(3): 258-266.

Herrmann, R.M., and K.V. Kowallik. 1970. Selective presentation of DNA-regions and membranes in chloroplast and mitochondria. *J. Cell Biol.* 45: 198-202.

Herrmann, F., and A. Meister. 1972. Separation and spectroscopical properties of pigment-protein complexes in *Antirrhinum* chloroplasts. *Photosynthetica*, 6: 177-182.

Heslop-Harrison, J. 1963. Structure and morphogenesis of lamellar systems in grana-containing chloroplasts. I. Membrane structure and lamellar architecture. *Planta*, 60: 243-260.

Highkin, H.R., N.K. Boardman, and D.J. Goodchild. 1969. Photosynthetic studies on a pea-mutant deficient in chlorophyll. *Plant Physiol.* 44: 1310-1320.

Holter, H. 1959. Pinocytosis. *Int. Rev. Cytol.* 8: 481-504. Academic Press, Inc., N.Y.

Hoober, J.K. 1970. Sites of synthesis of chloroplast membrane polypeptides in *Chlamydomonas reinhardi y-1*. *J. Biol. Chem.* 245: 4327-4334.

Hoober, J.K. 1972. A major polypeptide of chloroplast membranes of *Chlamydomonas reinhardi*. Evidence for synthesis in the cytoplasm as soluble component. *J. Cell Biol.* 52: 84-96.

Hoober, J.K., and G. Blobel. 1969. Characterization of the chloroplastic and cytoplasmic ribosomes of *Chlamydomonas reinhardi*. *J. Mol. Biol.* 41: 121-138.

Hoober, J.K., and W.J. Stegeman. 1973. Control of synthesis of a major polypeptide of chloroplast membranes in *Chlamydomonas reinhardi*. *J. Cell Biol.* 56: 1-12.

Horak, A., and S. Zalik. 1973. Development of photosystem I and photosystem II activities in a virescent mutant of barley. *Plant Physiol.* Suppl. 51:

Howell, S.H., and E.N. Moudrianakis. 1967*a*. Hill reaction site in chloroplast membranes: Non-participation of the quantasome particles in photoreduction. *J. Mol. Biol.* 27: 323-333.

Howell, S.H., and E.N. Moudrianakis. 1967*b*. Function of the "Quantasome" in photosynthesis: Structure and properties of membrane-bound particles active in the dark reactions of photophosphorylation. *Proc. Nat. Acad. Sci.* (Wash.) 58: 1261-1269.

Hyde, B.B., A.J. Hodge, A. Khan, and M.L. Birnstiel. 1963. Studies on phytoferritin. I. Identification and localization. *J. Ultrastruct. Res.* 9: 248-258.

Inoué, S. 1964. Organization and function of the mitotic spindle. *In* Primitive Motile Systems. *Edited by* R.D. Allen and N. Kamoya, Academic Press, New York. pp. 549-598.

Ireland, H.M.M., and J.W. Bradbeer. 1971. Plastid development in primary leaves of *Phaseolus vulgaris*. The effect of D-threo and L-threo chloramphenicol on the light induced formation of enzymes of the photosynthetic carbon pathway. *Planta*, 96: 254-261.

Jennings, H.S. 1904. The movements and reactions of *amoeba*. *Car. Inst. of Wash.* Pub. No. 6. pp. 126-234.

Jensen, W.A. 1965. The ultrastructure and composition of the egg and central cell of cotton. *Amer. J. Bot.* 52: 781-797.

Jones, L.B., N. Nagabhushan, E.B. Tucker, and S. Zalik. 1973. Dissociation reassociation and phenylalanine incorporation by chloroplast and cytoplasmic wheat leaf ribosomes. *Can. J. Biochem.* In press.

Kannangara, C.G. 1969. The formation of ribulose-diphosphate carboxylase protein during chloroplast development in barley. *Plant Physiol.* 44: 1533-1537.

Kawashima, N., and S.G. Wildman. 1970. Fraction I protein. *Ann. Rev. Plant Physiol.* 21: 325-358.

Keck, R.W., R.A. Dilley, C.F. Allen, and S. Biggs. 1970*a*. Chloroplast composition and structure differences in a soybean mutant. *Plant Physiol.* 46: 692-698.

Keck, R.W., R.A. Dilley, and B. Ke. 1970*b*. Photochemical characteristics in a soyabean mutant. *Plant Physiol.* 46: 699-704.

Khan, A. 1968*a*. Developmental physiology of bean leaf plastids. II. Negative contrast electron microscopy of tubular membranes in the prolamellar bodies. *Plant Physiol.* 43: 1769-1780.

Khan, A. 1968*b*. Developmental physiology of bean leaf plastids. III. Tube transformation and protochlorophyll(ide) photoconversion by a flash irradiation. *Plant Physiol.* 43: 1781-1785.

Kirk, J.T.O. 1963. The deoxyribonucleic acid of broad bean chloroplasts. *Biochem. J.* 88: 45p.

Kirk, J.T.O. 1967. Chapters I, X and XIII. *In* The Plastids. *Edited by* J.T.O. Kirk and R.A.E. Tilney-Bassett. W.K. Freeman and Company. London and San Francisco.

Kirk, J.T.O. 1971. Chloroplast structure and biogenesis. *Ann. Rev. Biochem.* 40: 161-196.

Kirk, J.T.O. 1971*a*. Will the real chloroplast DNA please stand up? *In* Autonomy and Biogenesis of Mitochondria and Chloroplasts. *Edited by* N.K. Boardman, A.W. Linnane and R.M. Smillie. North Holland, Amsterdam, London, New York. pp. 267-276.

Kislev, N., H. Swift, and L. Bogorad. 1965. Nucleic acids of chloroplasts and mitochondria in Swiss chard. *J. Cell Biol.* 25: 327-344.

Klein, S. 1962. Phytylation of chlorophyllide and formation of lamellae in chloroplasts. *Nature,* 196: 992-993.

Klein, S.G., G. Bryan, and L. Bogorad. 1964. Early stages in the development of plastid fine structure in red and far-red light. *J. Cell Biol.* 22: 433-442.

Kleinkopf, G.E., R.C. Huffaker, and A. Matheson. 1970. A simplified purification and some properties of ribulose-1,5-diphosphate carboxylase from barley. *Plant Physiol.* 46: 204-207.

Laetsch, W.M. 1968. Chloroplast specialization in dicotyledons possessing the C_4-dicarboxylic acid pathway of photosynthetic CO_2 fixation. *Amer.*

J. Bot. 55: 875-883.

Laetsch, W.M. 1970. Comparative ultrastructure of chloroplasts in plants with C-4 pathway of carbon fixation. *Plant Physiol.* 46: Suppl. 22 (Abstract).

Laetsch, W.M., and I. Price. 1969. Development of the dimorphic chloroplasts of sugar cane. *Amer. J. Bot.* 56: 77-87.

Laetsch, W.M. and D.A. Stetler. 1965. Chloroplast structure and function in cultured tobacco tissue. *Amer. J. Bot.* 52: 798-804.

Lafleche, D., J.M. Bove, and J. Duranton. 1972. Localization and translocation of the protochlorophyllide holochrome during greening of etioplasts in *Zea mays*. *J. Cell Sci.* 40: 205-214.

Ledbetter, M.C., and K.R. Porter. 1963. A "microtubule" in plant cell. Fine structure. *J. Cell Biol.* 19: 239-250.

Levine, R.P. 1969. Analysis of photosynthesis using mutant strains of algae and higher plants. *Ann. Rev. Plant Physiol.* 20: 523-540.

Levine, R.P., and A. Paszewski. 1970. Chloroplast structure and function in *ac-20*, a mutant strain of *Chlamydomonas reinhardi*. II. Photosynthetic electron transport. *J. Cell Biol.* 44: 540-546.

Levine, R.P., and R.K. Togasaki. 1965. A mutant strain of *Chlamydomonas reinhardi* lacking ribulose diphosphate carboxylase activity. *Proc. Nat. Acad. Sci.* (U.S.A.) 53: 987-990.

Lockshin. A., and R.H. Burris. 1966. Solubilization and properties of chloroplast lamellar protein. *Proc. Nat. Acad. Sci.* 56: 1564-1570.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Luft, J.H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409-414.

Lyttleton, J.W., and P.O.P. Ts'O. 1958. The localization of fraction I protein of green leaves in the chloroplasts. *Arch. Biochem. Biophys.* 73:

120-126.

Machold, O. 1971. Lamellar proteins of green and chlorotic chloroplasts as affected by iron deficiency and antibiotics. *Biochim. Biophys. Acta*, 238: 324-331.

Machold, O., A. Meister, and K. Adler. 1971. Spektroskopische Eigenschaften von elektrophoretisch getrennten Chlorophyll-Protein-Komplexen der Photosysteme I und II aus *Vicia faba* und *Chlorella pyrenoidosa*. *Photosynthetica*, 5: 160-165.

Maclachlan, S. 1962. Microscopic and biochemical studies on a chlorophyll mutant of Gateway barley. Ph.D. thesis, University of Alberta, Edmonton.

Maclachlan, S., and S. Zalik. 1963. Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Can. J. Bot.* 41: 1053-1062.

Mani, R.S., and S. Zalik. 1970. Physicochemical studies of bean and wheat chloroplast structural protein. *Biochim. Biophys. Acta*, 200: 132-137.

Margulies, M.M. 1962. Effect of chloramphenicol on light dependent development of seedlings of *Phaseolus vulgaris* var. Black Valentine, with particular reference to development of photosynthetic activity. *Plant Physiol.* 37: 473-480.

Margulies, M.M. 1971. Concerning the sites of proteins of chloroplast ribosomes and of Fraction I protein (ribulose-1,5-diphosphate carboxylase). *Biochem. Biophys. Res. Commun.* 44: 539-545.

Margulies, M.M., and F. Parenti. 1968. *In vitro* protein synthesis by plastids of *Phaseolus vulgaris*. III. Formation of lamellar and soluble chloroplast protein. *Plant Physiol.* 43: 504-514.

Mayo, M.A., and E.C. Cocking. 1969. Pinocytic uptake of polystyrene latex particles by isolated tomato fruit protoplasts. *Protoplasma*, 68: 223-230.

Mego, J.L., and A.T. Jagendorf. 1961. Effect of light on growth of black valentine bean plastids. *Biochim. Biophys. Acta*, 53: 237-254.

Mendiola, L., and T. Akazawa. 1964. Partial purification and the enzymatic nature of fraction I protein in rice leaves. *Biochemistry*, 3: 174-179.

Menke, W. 1962. Structure and chemistry of plastids. *Ann. Rev. Plant Physiol.* 13: 27-44.

Menke, W. 1964. Feinbau und Entwicklung der Plastiden. *Ber. Deu. Bot. Ges.* 77: 340-354.

Menke, W., and E. Jordan. 1959. Über die Wirkung von Carboxypeptidase auf das lamellare Structurproteid des Chloroplasten von *Allium porrum. Z. Naturforsh.* 146: 234-240.

Miller, R.A. 1965. Physiological and biochemical studies of a mutant from Gateway barley. Ph.D. Thesis, University of Alberta, Edmonton.

Miller, R.A., and S. Zalik. 1965. Effect of light quality, light intensity and temperature on pigment accumulation in barley seedlings. *Plant Physiol.* 40: 569-574.

Millerd, A., D.J. Goodchild, and D. Spencer. 1969. Studies on a maize mutant sensitive to low temperature. II. Chloroplast structure, development and physiology. *Plant Physiol.* 44: 567-583.

Millerd, A., and J.R. McWilliams. 1968. Studies on a maize mutant sensitive to low temperature. I. Influence of temperature and light on the production of chloroplast pigments. *Plant Physiol.* 43: 1967-1972.

Moon, K.E., and E.O.P. Thompson. 1969. Subunits from reduced and S-carboxy methylated ribulose-diphosphate carboxylase (fraction I protein). *Aus. J. Biol. Sci.* 22: 463-470.

Moudrianakis, E.N., S.H. Howell, and A.E. Karu. 1968. Characterization of "Quantasome" and its role in photosynthesis. *In* Comparative Biochemistry and Biophysics of Photosynthesis". *Edited by* K. Shibata, A. Takamiya, A.T. Jagendorf, and R.C. Fuller. Univ. of Tokyo Press. and Park Press Pennsylvania. pp. 67-81.

von Mühlethaler, K. 1971. The ultrastructure of plastids. *In* Structure and Function of Chloroplasts. *Edited by* Martin Gibbs, Springer-Verlag, New York. Heidelberg, Berlin. pp. 7-34.

von Mühlethaler, K., and A. Frey-Wyssling. 1959. Entwicklung und Struktur der Proplastiden. *J. Biophys. Biochem. Cyto.* 6: 507-512.

von Mühlethaler, K., H. Moor, and J.W. Szarkowski. 1965. The ultrastructure of the chloroplast lamellae. *Planta*, 67: 305-323.

Nadler, K.D., and S. Granick. 1970. Controls of chlorophyll synthesis in barley. *Plant Physiol.* 46: 240-246.

Nadler, K.D., H.A. Herron, and S. Granick. 1972. Development of chlorophyll and Hill activity. *Plant Physiol.* 49: 388-392.

Newcomb, E.H. 1969. Plant microtubules. *Ann. Rev. Plant Physiol.* 20: 253-286.

Nichols, B.W., J.M. Stubbs, and A.T. James. 1967. The lipid composition and ultrastructure of normal developing and degenerating chloroplasts. *In* Biochemistry of Chloroplasts. *Edited by* T.W. Goodwin, Vol. II. Academic Press, New York, and London. pp. 677-690.

Niklowitz, W., and G. Drews. 1956. Beiträge zur Cytologie der Blaualgen Gom Mitteilung. Untersuchungen zur Substrucktur von *Phormidium uncinatum* Gom. *Arch. Microbiol.* 24: 134-146.

Ogawa, T., R. Kanai, and K. Shibata. 1968. Distribution of carotenoids in the two photochemical systems of higher plants and algae. *In* Comparative Biochemistry and Biophysics of Photosynthesis. *Edited by* K. Shibata, A. Takamiya, A.T. Jagendorf and R.C. Fuller. Univ. Press, Tokyo and Park Press, Pennsylvania. pp. 22-35.

Ogawa, T., F. Obata, and K. Shibata. 1966. Two pigment proteins in spinach chloroplasts. *Biochim. Biophys. Acta*, 112: 223-234.

Park, R.B., and J. Biggins. 1964. Quantasomes: Size and composition. *Science*, 144: 1009-1011.

Park, R.B., and A.O. Pfeifhofer. 1969*a*. Ultrastructural observations on deep-etched thylakoids. *J. Cell Sci.* 5: 299-311.

Park, R.B., and A.O. Pfeifhofer. 1969*b*. The effect of ethylene diaminetetra-acetate washing on the structure of spinach thylakoids. *J. Cell Sci.* 5: 313-319.

Park, R.B., and N.G. Pon. 1961. Correlation of structure with function in *Spinacea oleracea* chloroplasts. *J. Mol. Biol.* 3: 1-10.

Park, R.B., and N.G. Pon. 1963. Chemical composition and the substructure of lamellae isolated from *Spinacea oleracea* chloroplasts. *J. Mol. Biol.* 6: 105-114.

Park, R.B., and P.V. Sane. 1971. Distribution of function and structure in chloroplast lamellae. *Ann. Rev. Plant Physiol.* 22: 395-430.

Pickett-Heaps, J.D. 1968. Microtubule-like structures in growing plastids or chloroplasts of two algae. *Planta*, 81: 193-200.

Porter, K.R. 1966. Cytoplasmic microtubules and their function. *In* Principles of Bimolecular Organization. *Edited by* G.E.W. Wolstenholme and M.O. Connor. Churchill London. pp. 308-345.

Provasoli, L., S.H. Hutner, and I.J. Pinter. 1951. Destruction of chloroplasts by streptomycin. *Cold Spring Harbor Symp. Quant. Biol.* 16: 113-120.

Raven, H.P. 1970. Multiple origin for plastids and mitochondria. *Science*, 169: 641-643.

Remy, R. 1971. Resolution of chloroplast lamellar proteins by electrophoresis in polyacrylamide gels. Different patterns obtained with fractions enriched in either chlorophyll *a* or chlorophyll *b*. *FEBS Letters*, 13: 313-317.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212.

Ridley, S.M., and R.M. Leech. 1969. Chloroplast survival *in vitro*. *In* Progress in Photosynthetic Research. Vol. I. *Edited by* H. Metzner. Tübingen. pp. 229-244.

Ridley, S.M., and R.M. Leech. 1970. Division of chloroplasts in artificial environment. *Nature*, 227: 463-465.

Ris, H., and W. Plaut. 1962. Ultrastructure of DNA-containing areas in the chloroplast of *Chlamydomonas*. *J. Cell Biol.* 13: 383-391.

Röbbelen, G. 1956. Über die Protochlorophyllreduktion in einer Mutante von *Arabidopsis thaliana* (L) Heynh. *Planta*, 47: 532-546.

Rustad, R.C. 1964. The physiology of pinocytosis. *In* Recent Progress in Surface Science. Vol. II. *Edited by* J.F. Danielli, K.G.A. Pankhurst and A.C. Riddiford. Academic Press, New York and London. pp. 353-380.

Rutner, A.C., and M.D. Lane. 1967. Non identical subunits of ribulose diphosphate carboxylase. *Biochem. Biophys. Res. Commun.* 28: 531-537.

Rutner, A.C. 1970. Estimation of the molecular weight of ribulose diphosphate carboxylase subunits. *Biochem. Biophys. Res. Commun.* 39: 923-929.

Sager, R., and G.E. Palade. 1957. Structure and development of chloroplast in *Chlamydomonas*. I. In normal green cells. *J. Biophys. Biochem. Cyto.* 3: 463-488.

Sane, P.V., D.J. Goodchild, and R.B. Park. 1970. Characterization of chloroplast photosystem I and II separated by a non-detergent method. *Biochim. Biophys. Acta*, 216: 162-172.

Sane, P.V., and S. Zalik. 1968. Amino acid, sugar, and organic acid content during germination of Gateway barley and its chlorophyll mutant. *Can. J. Bot.* 46: 1479-1485.

Sane, P.V., and S. Zalik. 1970. Metabolism of acetate-2-^{14}C, glycine-2-^{14}C, leucine-U-^{14}C, and effect of δ-aminolevulinic acid on chlorophyll synthesis in Gateway barley and its mutant. *Can. J. Bot.* 48: 1171-1178.

Schmid, G.H. 1967. Photosynthetic capacity and lamellar structure in various chlorophyll-deficient plants. *J. Microsc.* (Paris) 6: 485-498.

Shibata, K. 1957. Spectroscopic studies on chlorophyll formation in intact leaves. *J. Biochem.* (Tokyo) 44: 147-173.

Shumway, L.K., and T.E. Weier. 1967. Chloroplast structure of Iojap maize. *Amer. J. Bot.* 54: 773-780.

Sironval, C., J.M. Michel, R. Bronchart, and E. Englert-Dujardin. 1969. On the 'primary' thylakoids of chloroplasts grown under flash regime. *In* Progress in Photosynthetic Research. Vol. I. *Edited by* H. Metzner. Tübingen. pp. 47-54.

Sironval, C., M.R. Michel-Wolwertz, and A. Madsen. 1965.

On the nature and possible functions of the 673 and 684 mμ forms *in vivo* of chlorophyll. *Biochem. Biophys. Acta*, 94: 344-354.

Sissakian, N.M., I.I. Fillippovich, E.N. Svetailo, and K.A. Aliyev. 1965. On the protein synthesizing system of chloroplasts. *Biochim. Biophys. Acta*, 95: 474-485.

Slack, C.R., M.D. Hatch, and D.J. Goodchild. 1969. Distribution of enzymes in mesophyll and parenchyma-sheath chloroplasts of maize leaves in relation to the C_4-dicarboxylic acid pathway of photosynthesis. *Biochem. J.* 114: 489-498.

Smillie, R.M., M. Dwyer, A. Grieve, and N.I. Tobin. 1967. Evidence for the synthesis *in vivo* of proteins of the Calvin cycle and of the photosynthetic electron transport pathway on chloroplast ribosomes. *Biochem. Biophys. Res. Commun.* 28: 604-610.

Smillie, R.M., N.S. Scott, and D. Graham. 1968. Biogenesis of chloroplasts: Roles of chloroplast DNA and chloroplast ribosomes. *In* Comparative Biochemistry and Biophysics of Photosynthesis. *Edited by* K. Shibata, A. Takamiya, A.T. Jagendorf and R.C. Fuller. University Press, Tokyo and Park Press, Pennsylvania. pp. 332-353.

Smith, J.H.C. 1952. Factors affecting transformation of protochlorophyll to chlorophyll. *Carnegie Inst. Wash.* Yearbook, 51: 151-153.

Smith, J.H.C., L.J. Durham, and C.F. Wurster. 1959. Formation and bleaching of chlorophyll in *albino* corn seedlings. *Plant Physiol.* 34: 340-345.

Sprey, B. 1968. Zur Feinstruktur des Plastidenstromas von *Hordeum vulgare* I. *Protoplasma*, 66: 469-478.

Srivastava, L.M., M. Vesk, and A.P. Singh. 1971. Effect of chloramphenicol on membrane transformations in plastids. *Can. J. Bot.* 49: 587-593.

Steinmann, E. 1952. An electron microscopic study of the lamellar structure of chloroplasts. *Exp. Cell. Res.* 3: 367-372.

Stephens, R.E. 1967. The mitotic apparatus. Physical and chemical characterization of the 22S protein component and its subunits. *J. Cell Biol.* 32: 255-275.

Stephansen, K., and S. Zalik. 1971. Inheritance and qualitative analysis of pigments in a barley mutant. *Can. J. Bot.* 49: 49-51.

Steward, P.C., and J.T. Barber. 1964. The use of acrylamide gel electrophoresis in the investigation of the soluble proteins of the plants. *Ann. N.Y. Acad. Sci.* 121: (Art. 2) 525-531.

Tae, H. Ji., J. L. Hess, and A.A. Benson. 1968. Studies on chloroplast membrane structure. I. Association of pigments with chloroplast lamellar protein. *Biochim. Biophys. Acta*, 150: 676-685.

Tageeva, S.V., I.P. Generosova, and G.A. Semeyenova. 1969. The variety of ultrastructural organization of chloroplasts depending on the functional state of plant tissues and organs and genetic factors. *In* Progress in Photosynthetic Research. Vol. I. *Edited by* H. Metzner, Tübingen. pp. 21-35.

Tewari, K.K. 1971. Genetic autonomy of extranuclear organelles. *Ann. Rev. Plant Physiol.* 22: 141-168.

Tewari, K.K., and S.G. Wildman. 1966. Chloroplast DNA from tobacco leaves. *Science*, 153: 1269-1271.

Tewari, K.K., and S.G. Wildman. 1967. DNA polymerase in isolated tobacco chloroplasts and nature of the polymerised product. *Proc. Nat. Acad. Sci. U.S.* 58: 689-696.

Thornber, J.P., P.F. Gregory, C.A. Smith, and J.L. Bailey. 1967*a*. Studies on the nature of the chloroplast lamellae. I. Preparation and properties of some chlorophyll-protein complexes. *Biochemistry*, 6: 391-396.

Thornber, J.P., J.C. Stewart, M.W.C. Hatton, and J.L. Bailey. 1967*b*. Studies on the nature of chloroplast lamellae. II. Chemical composition and further physical properties of two chlorophyll protein complexes. *Biochemistry*, 6: 2006-2014.

Thornber, J.P., S.M. Ridley, and J.L. Bailey. 1966. Some observations on the Fraction I protein of *Beta vulgaris*. *In* Biochemistry of Chloroplasts. Vol. I. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 275-284.

Tilney, L.G. 1968. II. Ordering of subcellular units. The assembly of microtubules and their role on development of cell form. *Devl. Biol.* Suppl. 2: 63-102.

Tilney, L.G., and K.R. Porter. 1967. Studies on the microtubules in *Helizoa* II. *J. Cell Biol.* 34: 327-343.

Trown, P.W. 1965. An improved method for isolation of carboxy-dismutase. Probable identity with fraction I protein and the protein moiety of protochlorophyll-holochrome. *Biochemistry*, 4: 908-917.

Troxler, R.F., R. Lester, F.A. Croft, and J.T. Albright. 1969. Plastid development in albescent maize. *Plant Physiol.* 44: 1609-1618.

Vatter, A.E., and R.S. Wolfe. 1958. The structure of photosynthetic bacteria. *J. Bact.* 75: 480-488.

Veleminsky, J., and G. Röbbelen. 1966. Beziehungen von *Arabidopsis thaliana* (L.) HEYNH. *Planta*, 68: 15-35.

Vernon, L.P., E.R. Shaw, and B. Ke. 1966. A photochemically active particle derived from chloroplasts by the action of the detergent Triton X-100. *J. Biol. Chem.* 241: 4104-4109.

Vesk, M., F.V. Mercer, and J.V. Possingham. 1965. Observations on the origin of chloroplasts and mitochondria in leaf cells of higher plants. *Aus. J. Bot.* 13: 161-169.

Virgin, H.I., A. Khan, and D. von Wettstein. 1963. The physiology of chlorophyll formation in relation to structural changes in chloroplasts. *Photochem. Photobiol.* 2: 83-91.

Walker, G.W.R., J. Dietrich, R. Miller and K. Kasha. 1963. Recent barley mutants and their linkages. II. Genetic data for further mutants. *Can. J. Genet. Cyto.* 5: 200-219.

Walles, B. 1963. Macromolecular physiology of plastids. IV. On amino acid requirements of lethal chloroplast mutants in barley. *Hereditas*, 50: 317-344.

Walles, B. 1965. Plastid structure of carotenoid-deficient mutants of sunflower (*Helianthus annuus* L.). I. White mutant. *Hereditas*, 53: 247-256.

Walles, B. 1966. Plastid structure of carotenoid-deficient mutants of sunflower (*Helianthus annuus* L.). II. The yellow mutant. *Hereditas*, 56: 131-136.

Walles, B. 1967. Use of biochemical mutants in analyses of chloroplast morphogenesis. *In* Biochemistry of Chloroplasts. Vol. II. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 633-653.

Walles, B. 1971. Plastid inheritance and mutations. *In* Structure and Function of Chloroplasts. *Edited by* Martin Gibbs. Springer-Verlag New York. Heidelberg Berlin. pp. 51-88.

Walles, B. 1972. An electron microscope study on photodestruction of plastid ribosomes in β-carotene-deficient mutants of *Helianthus annuus*. *Protoplasma*, 75: 215-229.

Weber, P. 1962. Über lamellare Strukturproteide aus Chloroplasten verschiedener Pflanzen. *Z. Naturforsch.* 17b: 683-688.

Wehrmeyer, W. 1965. Zur Kristallgitter strucktur der sogenannten Prolamellarkörper in Proplastiden etiolierter Bohnen. *Z. Naturforsch.* 20b: 1270-1296.

Wehrmeyer, W., and G. Röbbelen. 1965. Räumliche Aspekte zur Membranschichtung in den Chloroplasten einer *Arabidopsis* Mutante unter Auswertung von Serienschnitten. III. Mitteilung über membrane Bildungsprozesse im Chloroplasten. *Planta*, 64: 105-128.

Weier, T.E., and A.A. Benson. 1966. The molecular nature of chloroplast membranes. *In* Biochemistry of Chloroplasts. Vol. I. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 91-113.

Weissbach, A., B.L. Horecker, and J. Hurwitz. 1956. The enzymatic formation of phosphoglyceric acid from ribulose diphosphate and carbon dioxide. *J. Biol. Chem.* 218: 795-810.

von Wettstein, D. 1958. The formation of plastid structure. *In* The Photochemical Apparatus, its Structure and Function. Brookhaven Symposium in Biol. 11: pp. 138-159.

von Wettstein, D., and A. Khan. 1960. Macromolecular physiology of plastids. *In* Europ. Reg. Conf. Electron Micro. *Edited by* A.L. Houwink and B.J. Spit. Delft: De nederlandse vereniging voor electronenmicroscopie. pp. 1051-1054.

von Wettstein, D. 1961. Nuclear and cytoplasmic factors in development of chloroplast structure and function. *Can. J. Bot.* 39: 1537-1544.

von Wettstein, D. 1966. On physiology of chloroplast structure. *In* Biochemistry of Chloroplasts. Vol. I. *Edited by* T.W. Goodwin. Academic Press, New York and London. pp. 19-21.

von Wettstein, D. 1967. Chloroplast structure and genetics. *In* Harvesting the Sun. *Edited by* A. San Pietro, A.G. Frances, and J.A. Thomas. Academic Press, New York and London. pp. 150-190.

von Wettstein, D., K.W. Henningsen, J.E. Boynton, G.C. Kannangara, and Q.F. Nielsen. 1971. The genetics of chloroplast development in barley. *In* Autonomy and Biogenesis of Mitochondria and Chloroplasts. *Edited by* N.K. Boardman, A.W. Linnane, and R.M. Smillie. North-Holland, Amsterdam and London, New York. pp. 205-233.

Wheeler, H., B.L. Baker, and P. Henchey. 1972. Pinocytosis in root cap cells exposed to uranyl salts. *Amer. J. Bot.* 59: 858-868.

Whaley, W.G., Kephart, J.E., and H.H. Mollenhauer. 1964. The dynamics of cytoplasmic membranes during development. *In* Cellular Membranes in Development. *Edited by* M. Locke. Academic Press, New York and London. pp.

Wildman, S.G., and J. Bonner. 1947. The proteins of green leaves. I. Isolation, enzymatic properties and auxin content of spinach cytoplasmic proteins. *Arch. Biochem. Biophys.* 14: 381-413.

Wildman, S.G., T. Honglandarom, and S.I. Honda. 1962. Chloroplasts and mitochondria in living plant cells: Cinephotomicrographic studies. *Science*, 138: 434-436.

Wolff, J.B., and L. Price. 1957. Terminal steps of chlorophyll a biosynthesis in higher plants. *Arch. Biochem. Biophys.* 72: 293-310.

Wolff, J.B., and L. Price. 1960. Effect of sugars on chlorophyll biosynthesis in higher plants. *J. Biol. Chem.* 235: 1603-1608.

Woodcock, C.L.F., and H. Fernandez-Moran. 1968. Electron microscopy of DNA confirmation in spinach chloroplasts. *J. Mol. Biol.* 31: 627-631.

Wrigley, C.W., H.L. Webster, and J.F. Turner. 1966. Electrophoresis of soluble proteins of wheat leaves. *Nature*, 209: 1133-1134.

Yokomura, E. 1967. An electron microscope study of DNA-like fibrils in chloroplasts. *Cytologia* (Tokyo), 32: 361-377.

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